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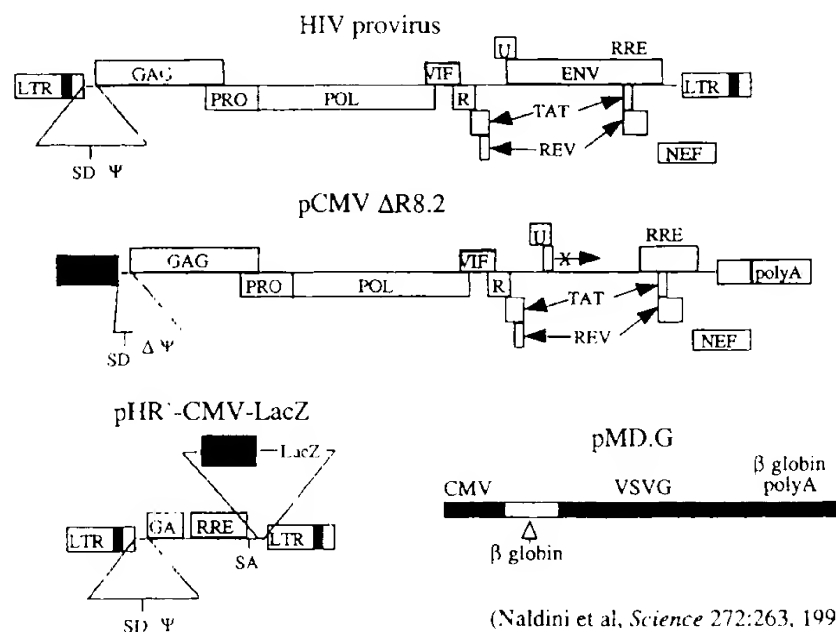
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(54) Title: PACKAGING CELL LINES FOR HIV-DERIVED RETROVIRAL VECTOR PARTICLES



(57) Abstract

Novel packaging cell lines useful for generating viral accessory protein independent HIV-derived retroviral vector particles, methods of constructing such packaging cell lines and methods of using the viral accessory protein independent HIV-derived retroviral vector particles are disclosed.

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PACKAGING CELL LINES FOR HIV-DERIVED RETROVIRAL VECTOR PARTICLES

BACKGROUND OF THE INVENTION

Retroviral vectors based on lentiviruses, such as human immunodeficiency viruses (HIV), can infect nondividing cells, and integration of proviral DNA occurs
5 without the need for cell division. These properties make lentiviruses attractive for gene transfer into nondividing cells, such as hepatocytes, myofibers, hematopoietic stem cells, and neurons.

However, the use of lentivirus vectors, particularly HIV vectors, particularly for gene therapy, is hampered by concern over their safety. Thus, a need for the
10 development of lentivirus vectors, particularly HIV vectors, with improved safety, particularly for gene therapy, exists.

SUMMARY OF THE INVENTION

The present invention relates to novel packaging cell lines useful for generating viral accessory protein independent lentivirus-derived, particularly HIV-derived,
15 retroviral vector particles, to construction of such cell lines and to methods of using the accessory protein independent lentivirus-derived retroviral vector particles to introduce DNA of interest into cells (e.g., eukaryotic cells such as animal (particularly mammalian), plant or yeast cells or prokaryotic cells such as bacterial cells). In a preferred embodiment, the packaging cell lines of the present invention are stable
20 packaging cell lines.

In one embodiment of the invention, packaging cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); and (b) a retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has

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been codon optimized by mutagenesis to improve expression of the lentivirus *gagpol* proteins.

In second embodiment of the invention, packaging cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles
5 comprise (a) a cell (e.g., mammalian cell); (b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been codon optimized by mutagenesis to improve expression of the lentivirus *gagpol* proteins; and (c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.

10 In a third embodiment of the invention, packaging cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been codon optimized by mutagenesis to improve expression of the lentivirus *gagpol*
15 proteins; (c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and (d) a third retroviral nucleotide sequence which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

In a fourth embodiment of the invention, packaging cell lines for producing a
20 viral accessory protein independent lentivirus-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been codon optimized by mutagenesis to improve expression of the lentivirus *gagpol* proteins; and (c) a retroviral nucleotide sequence which comprises a
25 DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

In a fifth embodiment of the invention, packaging cell lines for producing a viral accessory protein independent HIV-derived retroviral vector particles comprise (a) a cell

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(e.g., mammalian cell); and (b) a retroviral nucleotide sequence in the cell which comprises a coding sequence for HIV *gagpol*, wherein said coding sequence has been codon optimized by mutagenesis to improve expression of the HIV *gagpol* proteins.

In sixth embodiment of the invention, packaging cell lines for producing a viral
5 accessory protein independent HIV-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for HIV *gagpol*, wherein said coding sequence has been codon optimized by mutagenesis to improve expression of the HIV *gagpol* proteins; and
10 (c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.

In a seventh embodiment of the invention, packaging cell lines for producing a viral accessory protein independent HIV-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for HIV *gagpol*, wherein said coding sequence has been
15 codon optimized by mutagenesis to improve expression of the HIV *gagpol* proteins; (c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and (d) a third retroviral nucleotide sequence which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

20 In a eighth embodiment of the invention, packaging cell lines for producing a viral accessory protein independent HIV-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a retroviral nucleotide sequence in the cell which comprises a coding sequence for HIV *gagpol*, wherein said coding sequence has been codon optimized by mutagenesis to improve expression of the HIV *gagpol* proteins; and
25 (c) a retroviral nucleotide sequence which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

Alternatively, each of the packaging cell lines described herein can be produced using (1) a retroviral nucleotide sequence which comprises a codon optimized gag

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coding sequence and (2) a retroviral nucleotide sequence which comprises a codon optimized pol coding sequence, in place of the retroviral nucleotide sequence which comprises a codon optimized gagpol coding sequence.

In a particular embodiment, the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G). In another embodiment, the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus (MLV).

Cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles are produced by transfecting host cells (e.g., mammalian host cells) with a plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenesis to improve expression of the lentivirus *gagpol* proteins. Depending upon the particular cell line being produced, the host cells are also co-transfected with a plasmid comprising a DNA sequence which encodes a heterologous envelope protein, or a plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration, or both of these plasmids. Alternatively, host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a lentivirus *gag* protein and a plasmid comprising a codon optimized DNA sequence encoding a lentivirus *pol* protein, in place of the plasmid comprising a codon optimized DNA sequence encoding both lentivirus *gagpol* proteins.

Cell lines for producing a viral accessory protein independent HIV-derived retroviral vector particles are produced by co-transfecting host cells (e.g., mammalian host cells) with a plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenesis to improve expression of the HIV *gagpol* proteins. Depending upon the particular cell line being produced, the host cells are also co-transfected with a plasmid comprising a DNA sequence which encodes a heterologous envelope protein, or a plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse

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transcription and integration, or both of these plasmids. Alternatively, host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a HIV gag protein and a plasmid comprising a codon optimized DNA sequence encoding a HIV pol protein, in place of the plasmid comprising a codon optimized DNA sequence
5 encoding both HIV gagpol proteins.

The present invention also relates to methods of producing viral accessory protein independent lentivirus-derived retroviral vector particles, comprising co-transfecting host cells (e.g., mammalian host cells) with (a) a first plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence
10 has been codon optimized by mutagenesis to improve expression of the lentivirus gagpol proteins; (b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and (c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration. Alternatively, host cells are transfected with a plasmid comprising a
15 codon optimized DNA sequence encoding a lentivirus gag protein and a plasmid comprising a codon optimized DNA sequence encoding a lentivirus pol protein, in place of the first plasmid comprising a codon optimized DNA sequence encoding both lentivirus gagpol proteins.

In a particular embodiment, the invention relates to methods of producing viral
20 accessory protein independent HIV-derived retroviral vector particles, comprising co-transfecting host cells (e.g., mammalian host cells) with (a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenesis to improve expression of the HIV gagpol proteins; (b) a second plasmid comprising a DNA sequence which encodes a
25 heterologous envelope protein; and (c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration. Alternatively, host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a HIV gag protein and a plasmid comprising a

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codon optimized DNA sequence encoding a HIV pol protein, in place of the first plasmid comprising a codon optimized DNA sequence encoding both HIV gagpol proteins.

The present invention also relates to viral accessory protein-independent retroviral particles produced by or obtainable by (obtained by) the methods described herein.

The present invention further relates to isolated DNA encoding a codon optimized lentivirus *gagpol*, isolated DNA encoding the *gag* coding region of a codon optimized lentivirus *gagpol*, and isolated DNA encoding the *pol* coding region of a codon optimized lentivirus *gagpol*. In a particular embodiment, the present invention relates to isolated DNA encoding a codon optimized HIV *gagpol*, isolated DNA encoding the *gag* coding region of a codon optimized HIV *gagpol*, and isolated DNA encoding the *pol* coding region of a codon optimized HIV *gagpol*.

The packaging cell lines and viral particles of the present invention can be used for gene therapy or gene replacement with improved safety. The packaging cell lines and viral particles of the present invention can also be used in development and production of vaccines, and in production of biochemical reagents. Gene therapy vectors produced with the cell lines of the present invention are expected to be valuable medical therapeutics.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram of an expression cassette containing the codon optimized *gagpol* genes. The DNA was constructed in multiple segments, which are indicated at the top as 1/3, 2/3, 3/3 (A, B, C and D) and HIN. Restriction sites used to assemble the cloned segments are indicated above the kilobasepair (Kb) ruler. Below the ruler are multiple features showing the location of the human cytomegalovirus (CMV) promoter, human betaglobin sequences (Bglobin), mRNA sequences (thinner line represents intronic sequence), the *gag* and *pol* open reading frames, the individual

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proteolytic fragment coding sequences (p17_MA, p24_CA, p7, p6, PR, p51_RT, RNaseH and integrase (IN)) and each synthetic oligonucleotide used in the assembly process (multiple adjacent open arrows).

Figure 2 is a table which depicts codon usage frequencies in genes which are highly expressed and in the codon optimized gagpol open reading frame of the HIV packaging construct described herein.

Figure 3 is a schematic representation of the HIV provirus and a three-plasmid expression system used for generating a pseudotyped HIV-based vector by transient transfection as described in Naldini *et al.*, *Science*, 272:263-267 (1996).

Figure 4 is a list of some characteristics relating to the HIV Rev protein.

Figure 5 is a list of some points relating to codon optimization of HIV gagpol.

Figure 6 is a partial DNA sequence of HIV gag (SEQ ID NO: 1), showing inactivation of inhibitory sequences as described in Schwartz, S. *et al.*, *J. Virol.*, 66(12):7176-7182 (1992).

Figure 7 a plot of the %(G+C) content of wildtype HIV gagpol sequences and theoretically codon optimized HIV gagpol sequences. The percent of bases, either G or C, was calculated for a 30 nucleotide moving window for the entire length of the gagpol gene, and the value plotted versus nucleotide position. Diamonds = HIV gagpol sequences; squares = full optimal back-translation for gag open reading frame; triangles = full optimal back-translation for pol open reading frame; CO = codon optimized.

Figures 8A-8E depict the alignment of the nucleotide sequences and predicted amino acid sequences for the gag coding region of a wildtype HIV gagpol and a codon optimized HIV gagpol. "NL4-3 genbank.SEQ" indicates the nucleotide sequence (SEQ ID NO:2) and predicted amino acid sequence (SEQ ID NO:3) for the gag coding region of a wildtype HIV gagpol. "pHDMHgm2.seq" indicates the nucleotide sequence (SEQ ID NO:4) and predicted amino acid sequence (SEQ ID NO:5) for the gag coding region

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of a codon optimized HIV *gagpol*. The "NL4-3 genbank.SEQ" sequences are publicly available at the NIH GenBank sequence repository (Accession No. M19921).

Figures 9A-9L depict the alignment of the nucleotide sequences and predicted amino acid sequences for the *pol* coding region of a wildtype HIV *gagpol* and a codon optimized HIV *gagpol*. "NL4-3 genbank.SEQ" indicates a nucleotide sequence (SEQ ID NO:6) and a predicted amino acid sequence (SEQ ID NO:7) for the *pol* coding region of a wildtype HIV *gagpol* available in the NIH GenBank sequence repository (Accession No. M19921). The nucleotide and amino acid sequences for the *pol* coding region available in the GenBank sequence repository contain two sequence errors, which are indicated in Figures 9A-9L with shading. "pNL4-3.seq" indicates the correct nucleotide sequence (SEQ ID NO:8) and predicted amino acid sequence (SEQ ID NO:9) for the *pol* coding region of a wildtype HIV *gagpol*. "pHDMHgpm2.seq" indicates the nucleotide sequence (SEQ ID NO:10) and predicted amino acid sequence (SEQ ID NO:11) for the *pol* coding region of a codon optimized HIV *gagpol*.

Figures 10A-10D depict the DNA sequence (SEQ ID NO:12) for pHDMHgpm2. The CMV enhancer/promoter is at nucleotides 97 to 679, human betaglobin sequences (Bglobin) are at nucleotides 761 to 864, 865 to 1303 and 5710 to 6469 (end of Bglobin is at nucleotides 6445 to 6469), mRNA sequences are at nucleotides 680 to 778 and 1255 to 5921, SV40 origin of replication is at nucleotides 8796 to 8908, beta-lactamase (*bla*) coding region is at nucleotides 6709 to 7569, intron sequences are at nucleotides 779 to 1254, the codon optimized *gag* coding region is at nucleotides 1318 to 2820, the codon optimized *pol* coding region is at nucleotides 2619 to 5624 and the poly A site is at nucleotides 5897 to 5921.

Figure 11 is a circular map of plasmid pHDMHgpm2.

25 DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel packaging cell lines useful for generating viral accessory protein independent lentivirus-derived, particularly HIV-derived,

retroviral vector particles, to construction of such cell lines and to methods of using the accessory protein independent lentivirus-derived retroviral vector particles to introduce DNA of interest into cells (e.g. eukaryotic cells such as animal (particularly mammalian), plant or yeast cells or prokaryotic cells such as bacterial cells). In a particular embodiment, the packaging cell lines of the present invention are stable packaging cell lines.

The cell lines are engineered to express the lentivirus proteins necessary for virus particle formation (*gagpol* proteins), without containing DNA sequences from lentivirus accessory proteins (*tat*, *vif*, *vpr*, *vpu*, *nef* and *rev* proteins and Rev response element (RRE)). Additionally, no viral sequences (such as cis-acting elements termed constitutive transport elements (CTEs)) will be expressed as RNA of any kind. DNA sequences for lentivirus *gagpol* are codon optimized by extensively mutagenizing the sequences to improve expression and to reduce the risk of recombination between transfer vector sequences and *gagpol* messenger RNA. This greatly improves the safety of virus preparations generated from these cell lines. In a particular embodiment, the DNA sequences for lentivirus *gagpol* are not codon optimized in the overlap region between the *gag* and *pol* sequences and in cis-acting signals necessary for translation of *pol*.

Examples of lentiviruses include human immunodeficiency viruses (e.g., HIV-1, HIV-2, HIV-3), bovine lentiviruses (e.g., bovine immunodeficiency viruses, bovine immunodeficiency-like viruses, Jembrana disease viruses), equine lentiviruses (e.g., equine infectious anemia viruses), feline lentiviruses (e.g., feline immunodeficiency viruses, panther lentiviruses, puma lentiviruses), ovine/caprine lentiviruses (e.g., Brazilian caprine lentiviruses, caprine arthritis-encephalitis viruses, Maedi-Visna viruses, Maedi-Visna-like viruses, Maedi-Visna-related viruses, ovine lentiviruses, Visna lentiviruses), Simian AIDS retroviruses (e.g., human T-cell lymphotropic virus type 4), simian immunodeficiency viruses, simian-human immunodeficiency viruses, human lymphotropic viruses (e.g., type III), simian T-cell lymphotropic viruses.

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In another embodiment, cell lines are engineered to express the HIV proteins necessary for virus particle formation (*gagpol* proteins), without containing DNA sequences from HIV accessory proteins (*tat*, *vif*, *vpr*, *vpu*, *nef* and *rev* proteins and Rev response element (RRE)). Additionally, no viral sequences (such as cis-acting elements
5 termed constitutive transport elements (CTEs)) will be expressed as RNA of any kind. DNA sequences for a HIV *gagpol* are codon optimized by mutagenesis to improve expression and to reduce the risk of recombination between transfer vector sequences and *gagpol* messenger RNA. In a particular embodiment, the DNA sequences for HIV *gagpol* are not codon optimized in the overlap region between the *gag* and *pol*
10 sequences and in cis-acting signals necessary for translation of *pol*.

Alternatively, each of the packaging cell lines described herein can be produced using (1) a nucleotide sequence which comprises a codon optimized *gag* coding sequence and (2) a nucleotide sequence which comprises a codon optimized *pol* coding sequence, in place of the nucleotide sequence which comprises a codon optimized
15 *gagpol* coding sequence. In this embodiment, the *gag* and *pol* coding sequences can be completely codon optimized

Benefits of the present invention include the removal of potentially harmful lentivirus accessory proteins and other viral sequences, and the reduction of the risk of recombination to produce replication competent virus.

20 Packaging cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles comprise a mammalian cell and a retroviral nucleotide sequence comprising a coding sequence for a lentivirus *gagpol* which has been codon optimized. In a particular embodiment the packaging cell lines further comprise a retroviral nucleotide sequence comprising a coding sequence for a
25 heterologous envelope protein. In a second embodiment, the packaging cell lines further comprise a retroviral nucleotide sequence comprising a coding sequence for a heterologous envelope protein and a retroviral nucleotide sequence which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse

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transcription and integration. In third embodiment, the packaging cell lines further comprise a retroviral nucleotide sequence which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration. Alternatively, the packaging cell lines of the present invention comprise a
5 retroviral nucleotide sequence which comprises a codon optimized gag coding sequence and (2) a retroviral nucleotide sequence which comprises a codon optimized pol coding sequence, in place of the retroviral nucleotide sequence which comprises a codon optimized gagpol coding sequence.

The coding sequence(s) for lentivirus *gagpol* which has (have) been codon
10 optimized results in improved expression of the lentivirus *gagpol* proteins and reduces the risk of recombination between the transfer vector and *gagpol* messenger RNA. Codon optimization of the coding sequence(s) for lentivirus *gagpol* was obtained by mutagenizing for each particular amino acid residue, specific nucleic acid bases in a codon for the particular amino acid residue to a nucleic acid base which is present in a
15 codon which occurs at a high frequency in genes which are highly expressed for the same amino acid residue. In a particular embodiment, the resulting optimized codon also does not cause introduction of mRNA splicing signals into the codon optimized sequence. Thus, in a particular embodiment, codon optimization of the coding sequence(s) for lentivirus *gagpol* is obtained by mutagenizing for each particular amino
20 acid residue, specific nucleic acid bases in a codon for the particular amino acid residue to a nucleic acid base that is present in a codon which (1) occurs at a high frequency in genes which are highly expressed for the same amino acid residue and (2) does not cause introduction of mRNA splicing signals into the codon optimized sequence. Codon optimization typically results in the removal of nucleic acid base A-rich
25 instability elements.

In a particular embodiment, the coding sequence for a HIV *gagpol* (pNL4-3; available through the AIDS repository, NIH; Adachi *et al.*, *J. Virol.*, 59:284-291 (1986)) has been codon optimized to improve translational efficiency of the HIV *gagpol*

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proteins and reduce the risk of recombination between the transfer vector and HIV gagpol messenger RNA. Two hundred thirty-seven base pairs (237 bp) consisting of the gag pol overlap and cis-acting signals necessary for translation of pol (nucleotides 2583 to 2819 of SEQ ID NO: 12) were not optimized. The HIV *gagpol* sequence obtained using the codon optimization process does not differ at the amino acid level from the wildtype HIV *gagpol* sequence, but differs at the nucleotide level from the HIV *gagpol* sequence. A codon optimized HIV *gag* sequence is shown in Figures 8A-8E (pHDMHgpm2.seq) (SEQ ID NO:4). A codon optimized HIV *pol* sequence is shown in Figures 9A-9L (pHDMHgpm2.seq) (SEQ ID NO:10).

10 A plasmid comprising DNA sequences which encode codon optimized lentivirus *gagpol* proteins is also referred to herein as a packaging construct. This plasmid includes a promoter which drives the expression of the *gagpol* proteins, such as the human cytomegalovirus (hCMV) immediate early promoter. This plasmid is defective for the production of the viral envelope and accessory proteins tat, vif, vpr, vpu, nef and rev and the Rev response element (RRE). The packaging construct also does not contain viral sequences which are transcribed into mRNA, such as constitutive transport elements (CTEs).

A packaging construct comprising a codon optimized HIV *gagpol* is depicted in Figure 1 and in Figure 11. Figures 10A-10D depict the DNA sequence (SEQ ID NO:12) for the packaging construct pHDMHgpm2. This packaging construct (pHDMHgpm2) was constructed as follows: Plasmid pMDA.HIVgp mam was generated by chemical synthesis and PCR assembly (which is described in, for example, Stemmer *et al.*, *Gene*, 164:49-53 (1995)) of 215 different oligonucleotides. The DNA sequence for pMDA.HIVgp mam is the same as the DNA sequence for pMDA.HIVgp jtg except for 4.3 kb which was codon optimized using the DNASTar program (LaserGene, Madison, WI). Two hundred thirty-seven base pairs (237 bp) consisting of the gag pol overlap and cis-acting signals necessary for translation of pol (nucleotides 2583 to 2819 of SEQ ID NO: 12) were not optimized due to dual reading

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frame constraints. A NsiI site 5' of IN was preserved to aid fusion with wildtype sequences. Several single or double base pair silent mutations were introduced either to prevent potential splice donors and acceptors, or by the synthesis process.

pMDA.HIVgp jtg was derived from HIV-1 strain NL4-3. The protease mutation that is present in the NL4-3 NIH GenBank sequence was then repaired (Figure 9B), changing the nucleotide present at position 2948 of SEQ ID NO:12 from a "G" to a "C", thereby producing the codon present at nucleotide positions 2948 to 2950 of SEQ ID NO:12 which encodes an arginine instead of the glycine present in the NL4-3 GenBank amino acid sequence. The resulting plasmid was named pMDHgpmam. The EcoRI-HindIII
10 fragment of pMDHgpmam was inserted into pHDM2b, a high copy version of the pMD vector (Ory, D. *et al.*, *Proc. Natl. Acad. Sci. USA*, 93(21):11400-11406 (1996)), to produce plasmid pHDMHgpm. The sequencing mutation that is present in the RNase domain of the NL4-3 NIH GenBank sequence was repaired (Figure 9H), changing the codon present at nucleotide positions 4724 to 4726 of SEQ ID NO:12 from "GGG" to
15 "AAG", thereby producing a codon encoding a lysine instead of the glycine present in the NL4-3 GenBank amino acid sequence. The resulting plasmid was named pHDMHgpm2. Codon usage frequencies in the codon optimized gagpol open reading frame of the packaging construct pHDMHgpm2 are shown in Figure 2.

As used herein, a heterologous envelope protein permits pseudotyping of
20 particles generated by the packaging construct and includes the G glycoprotein of vesicular stomatitis virus (VSV G) and the amphotropic envelope of the Moloney leukemia virus (MLV). A plasmid comprising a DNA sequence which encodes a heterologous envelope protein is also referred to herein as an envelope coding plasmid.

The terms "mammal" and "mammalian", as used herein, refer to any vertebrate
25 animal, including monotremes, marsupials and placental, that suckle their young and either give birth to living young (eutharian or placental mammals) or are egg-laying (metatharian or nonplacental mammals). Examples of mammalian species include

humans and other primates (e.g., monkeys, chimpanzees), rodents (e.g., rats, mice, guinea pigs) and ruminants (e.g., cows, pigs, horses).

Examples of mammalian cells include human (such as HeLa cells, 293T cells, NIH 3T3 cells), bovine, ovine, porcine, murine (such as embryonic stem cells), rabbit and monkey (such as COS1 cells) cells. The cell may be a non-dividing cell (including hepatocytes, myofibers, hematopoietic stem cells, neurons) or a dividing cell. The cell may be an embryonic cell, bone marrow stem cell or other progenitor cell. Where the cell is a somatic cell, the cell can be, for example, an epithelial cell, fibroblast, smooth muscle cell, blood cell (including a hematopoietic cell, red blood cell, T-cell, B-cell, etc.), tumor cell, cardiac muscle cell, macrophage, dendritic cell, neuronal cell (e.g., a glial cell or astrocyte), or pathogen-infected cell (e.g., those infected by bacteria, viruses, virusoids, parasites, or prions).

Typically, cells isolated from a specific tissue (such as epithelium, fibroblast or hematopoietic cells) are categorized as a "cell-type." The cells can be obtained commercially or from a depository or obtained directly from an animal, such as by biopsy. Alternatively, the cell need not be isolated at all from the animal where, for example, it is desirable to deliver the virus to the animal in gene therapy.

To produce the cell lines of the present invention for producing a viral accessory protein independent lentivirus-derived retroviral vector particles, mammalian host cells are co-transfected with (a) a first plasmid comprising DNA sequence which encode lentivirus *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenesis, as described above, to improve expression of the lentivirus *gagpol* proteins; and (2) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein, or a retroviral nucleotide sequence which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration, or both, under conditions appropriate for transfection of the cells.

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In a particular embodiment, to produce the cell lines of the present invention for producing viral accessory protein independent HIV-derived retroviral vector particles mammalian host cells were cotransfected with (a) a first plasmid comprising DNA sequence which encode HIV *gagpol* proteins, wherein said DNA sequence has been
5 codon optimized by mutagenesis, as described above, to improve expression of the HIV *gagpol* proteins; and (2) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein, or a retroviral nucleotide sequence which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration, or both, under conditions appropriate for transfection of
10 the cells.

Virus stocks consisting of viral accessory protein independent lentivirus-derived, particularly HIV-derived, retroviral vector particles of the present invention are produced by maintaining the transfected cells under conditions suitable for virus production (e.g., in an appropriate growth media and for an appropriate period of time).
15 Such conditions, which are not critical to the invention, are generally known in the art. See, e.g., *Sambrook et al., Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor University Press, New York (1989); *Ausubel et al., Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1998); U.S. Patent No. 5,449,614; and U.S. Patent No. 5,460,959, the teachings of which are incorporated
20 herein by reference.

To generate viral accessory protein independent lentivirus-derived retroviral vector particles, mammalian host cells can be co-transfected with (a) a first plasmid comprising DNA sequence which encode lentivirus *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenesis, as described above, to improve
25 expression of the lentivirus *gagpol* proteins; (b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and (c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration. Alternatively, mammalian cells are

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transfected with a plasmid comprising a codon optimized DNA sequence encoding a lentivirus gag protein and a plasmid comprising a codon optimized DNA sequence encoding a lentivirus pol protein, in place of the first plasmid comprising a codon optimized DNA sequence encoding both lentivirus gagpol proteins. Alternatively, 5 mammalian host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a lentivirus gag protein and a plasmid comprising a codon optimized DNA sequence encoding a lentivirus pol protein, in place of the first plasmid comprising a codon optimized DNA sequence encoding both lentivirus gagpol proteins.

In a particular embodiment, the invention relates to methods of producing viral 10 accessory protein independent HIV-derived retroviral vector particles, comprising co-transfecting mammalian host cells with (a) a first plasmid comprising DNA sequence which encode HIV *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenesis, as described above, to improve expression of the HIV gagpol proteins; (b) a second plasmid containing a DNA sequence which encodes a 15 heterologous envelope protein; and (c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration. Alternatively, mammalian host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a HIV gag protein and a plasmid comprising a codon optimized DNA sequence encoding a HIV pol protein, in 20 place of the first plasmid comprising a codon optimized DNA sequence encoding both HIV gagpol proteins.

Virus particles produced by the methods described herein, using a codon optimized HIV packaging construct produced as described herein, were compared by Western analysis with virus particles produced as described in Naldini *et al.*, *Science*, 25 272:263-267 (1996), using the packaging construct plasmid pCMVAR8.2. Both the immunological reactivity and the proteolytic processing were confirmed to be indistinguishable.

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A plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration is also referred to herein as a transfer vector. A transfer vector, as used herein, refers to a vehicle which is used to introduce a DNA of interest into a eukaryotic cell, particularly a mammalian cell.

5 Figure 3 depicts an example of a transfer vector.

DNA sequence of interest, as used herein, include all or a portion of a gene or genes encoding a nucleic acid product whose expression in a cell or a mammal is desired. In a particular embodiment, the nucleic acid product is a heterologous therapeutic protein. Examples of therapeutic proteins include antigens or immunogens, such as a polyvalent vaccine, cytokines, tumor necrosis factor, interferons, interleukins, adenosine deaminase, insulin, T-cell receptors, soluble CD4, growth factors, such as epidermal growth factor, human growth factor, insulin-like growth factors, fibroblast growth factors), blood factors, such as Factor VIII, Factor IX, cytochrome b, glucocerebrosidase, ApoE, ApoC, ApoA1, the LDL receptor, negative selection markers or "suicide proteins", such as thymidine kinase (including the HSV, CMV, VZV TK), anti-angiogenic factors, Fc receptors, plasminogen activators, such as t-PA, u-PA and streptokinase, dopamine, MHC, tumor suppressor genes such as p53 and Rb, monoclonal antibodies or antigen binding fragments thereof, drug resistance genes, ion channels, such as a calcium channel or a potassium channel, adrenergic receptors, hormones (including growth hormones) and anti-cancer agents. In another embodiment, the nucleic acid product is a gene product to be expressed in a cell or a mammal and which product is otherwise defective or absent in the cell or mammal. For example, the nucleic acid product can be a functional gene(s) which is defective or absent in the cell or mammal.

25 DNA sequence of interest includes DNA sequences (control sequences) which are necessary to drive the expression of the gene or genes. The control sequences are operably linked to the gene. The term "operably linked", as used herein, is defined to mean that the gene is linked to control sequences in a manner which allows expression

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of the gene (or the nucleic acid sequence). Generally, operably linked means contiguous.

Control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal
5 binding sites and sequences which control termination of transcription and translation. In a particular embodiment, a recombinant gene encoding a desired nucleic acid product can be placed under the regulatory control of a promoter which can be induced or repressed, thereby offering a greater degree of control with respect to the level of the product produced.

10 As used herein, the term "promoter" refers to a sequence of DNA, usually upstream (5') of the coding region of a structural gene, which controls the expression of the coding region by providing recognition and binding sites for RNA polymerase and other factors which may be required for initiation of transcription. Suitable promoters are well known in the art. Exemplary promoters include the SV40, CMV and human
15 elongation factor (EF1) promoters. Other suitable promoters are readily available in the art (see, e.g., Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York (1998); Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor University Press, New York (1989); and U.S. Patent No. 5,681,735).

20 A DNA sequence of interest can be isolated from nature, modified from native sequences or manufactured *de novo*, as described in, for example, Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1998); and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor University Press, New York (1989). DNA sequences can be isolated and fused
25 together by methods known in the art, such as exploiting and manufacturing compatible cloning or restriction sites.

The packaging cell lines and viral particles of the present invention can be used, *in vitro*, *in vivo* and *ex vivo*, to introduce DNA of interest into a eukaryotic cell (e.g., a

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mammalian cell) or a mammal (e.g., a human or other mammal or vertebrate). The cells can be obtained commercially or from a depository or obtained directly from a mammal, such as by biopsy. The cells can be obtained from a mammal to whom they will be returned or from another/different mammal of the same or different species. For example, using the packaging cell lines or viral particles of the present invention, DNA of interest can be introduced into nonhuman cells, such as pig cells, which are then introduced into a human. Alternatively, the cell need not be isolated from the mammal where, for example, it is desirable to deliver viral particles of the present invention to the mammal in gene therapy.

10 *Ex vivo* therapy has been described, for example, in Kasid *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:473 (1990); Rosenberg *et al.*, *N. Engl. J. Med.*, 323:570 (1990); Williams *et al.*, *Nature*, 310:476 (1984); Dick *et al.*, *Cell*, 42:71 (1985); Keller *et al.*, *Nature*, 318:149 (1985); and Anderson *et al.*, United States Patent No. 5,399,346.

Methods for administering (introducing) viral particles directly to a mammal are generally known to those practiced in the art. For example, modes of administration include parenteral, injection, mucosal, systemic, implant, intraperitoneal, oral, intradermal, transdermal (e.g., in slow release polymers), intramuscular, intravenous including infusion and/or bolus injection, subcutaneous, topical, epidural, etc. Viral particles of the present invention can, preferably, be administered in a pharmaceutically acceptable carrier, such as saline, sterile water, Ringer's solution, and isotonic sodium chloride solution.

The dosage of a viral particle of the present invention administered to a mammal, including frequency of administration, will vary depending upon a variety of factors, including mode and route of administration; size, age, sex, health, body weight and diet of the recipient mammal; nature and extent of symptoms of the disease or disorder being treated; kind of concurrent treatment, frequency of treatment, and the effect desired.

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The teachings of all the articles, patents, patent applications and GenBank sequences cited herein are incorporated by reference in their entirety.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that
5 various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

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CLAIMS

What is claimed is:

1. A packaging cell line for producing a viral accessory protein independent HIV-derived retroviral vector particle comprising:
 - 5 a) a mammalian cell;
 - b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a HIV *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the HIV *gagpol* proteins;
 - c) a second retroviral nucleotide sequence in the cell which comprises the
10 coding sequence for a heterologous envelope protein; and
 - d) a third retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.
2. A packaging cell line of Claim 1 wherein the heterologous envelope protein is
15 the G glycoprotein of vesicular stomatitis virus (VSV G).
3. A packaging cell line of Claim 1 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.
4. A packaging cell line of Claim 1 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
- 20 5. A packaging cell line comprising:
 - a) a mammalian cell;

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- b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a HIV *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the HIV *gagpol* proteins; and
 - c) a second retroviral nucleotide sequence in the cell which comprises a
5 DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.
6. A packaging cell line of Claim 5 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
7. A packaging cell line comprising:
- 10 a) a mammalian cell;
 - b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a HIV *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the HIV *gagpol* proteins; and
 - c) a second retroviral nucleotide sequence in the cell which comprises the
15 coding sequence for a heterologous envelope protein.
8. A method of producing a packaging cell line for producing a viral accessory protein independent HIV-derived retroviral vector particle, comprising co-transfecting mammalian host cells with:
- 20 a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the HIV *gag* and *pol* proteins;
 - b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and

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- c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.
9. A method of Claim 8 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).
10. A method of Claim 8 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.
11. A method of Claim 8 wherein the DNA sequence of interest is a heterologous therapeutic protein.
- 10 12. A method of producing a viral accessory protein independent HIV-derived retroviral vector particle comprising co-transfecting mammalian host cells with:
- a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the HIV *gagpol* proteins;
- 15 b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
- c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.
- 20 13. A method of Claim 12 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

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14. A method of Claim 12 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.
15. A method of Claim 12 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
- 5 16. A packaging cell line for producing a viral accessory protein independent lentivirus-derived retroviral vector particle comprising:
 - a) a mammalian cell;
 - b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a lentivirus *gagpol*, wherein said coding sequence
10 has been mutagenized to improve expression of the lentivirus gagpol proteins;
 - c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and
 - d) a third retroviral nucleotide sequence in the cell which comprises a DNA
15 sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.
17. A packaging cell line of Claim 16 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).
18. A packaging cell line of Claim 16 wherein the heterologous envelope protein is
20 the amphotropic envelope of the Moloney leukemia virus.
19. A packaging cell line of Claim 16 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

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20. A packaging cell line comprising:
- a) a mammalian cell;
 - b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the lentivirus *gagpol* proteins; and
 - c) a second retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.
- 10 21. A packaging cell line of Claim 20 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
22. A packaging cell line comprising:
- a) a mammalian cell;
 - b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the lentivirus *gagpol* proteins; and
 - c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.
- 15 23. A method of producing a packaging cell line for producing a viral accessory protein independent lentivirus-derived retroviral vector particle, comprising co-transfecting mammalian host cells with:
- a) a first plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the lentivirus *gag* and *pol* proteins;
- 25

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- b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
 - c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and -
5 integration.
24. A method of Claim 23 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).
25. A method of Claim 23 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.
- 10 26. A method of Claim 23 wherein the DNA sequence of interest is a heterologous therapeutic protein.
27. A method of producing a viral accessory protein independent lentivirus-derived retroviral vector particle comprising co-transfecting mammalian host cells with:
- a) a first plasmid comprising a DNA sequence which encodes lentivirus
15 *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the lentivirus *gagpol* proteins;
 - b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
 - c) a third plasmid comprising a DNA sequence of interest and lentivirus
20 cis-acting sequences required for packaging, reverse transcription and integration.
28. A method of Claim 27 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

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29. A method of Claim 27 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.
30. A method of Claim 27 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
- 5 31. A viral accessory protein independent HIV-derived retroviral vector particle produced by the method comprising co-transfecting mammalian host cells with:
- a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the HIV *gagpol* proteins;
 - 10 b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
 - c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.
- 15 32. A method of Claim 31 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).
33. A method of Claim 31 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.
34. A method of Claim 31 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
- 20

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35. A viral accessory protein independent lentivirus-derived retroviral vector particle produced by the method comprising co-transfecting mammalian host cells with:
- 5 a) a first plasmid comprising a DNA sequence which encodes lentivirus - *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the lentivirus *gagpol* proteins;
- b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
- 10 c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.
36. A method of Claim 35 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).
37. A method of Claim 35 wherein the heterologous envelope protein is the
15 amphotropic envelope of the Moloney leukemia virus.
38. A method of Claim 35 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
39. Isolated DNA encoding a codon optimized HIV *gagpol*.
40. Isolated DNA encoding a codon optimized HIV *gag*.
- 20 41. Isolated DNA of Claim 40 comprising the nucleotide sequence of SEQ ID NO:4.
42. Isolated DNA encoding a codon optimized HIV *pol*.

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43. Isolated DNA of Claim 42 comprising the nucleotide sequence of SEQ ID NO:10.
44. A method of introducing a DNA sequence of interest into a mammal comprising introducing into said mammal a viral accessory protein independent HIV-derived retroviral vector particle comprising the DNA sequence of interest.
45. The method of Claim 44 wherein the mammal is a human.
46. The method of Claim 44 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
47. A method of introducing a DNA sequence of interest into a mammal comprising the steps of:
- a) introducing into cells a viral accessory protein independent HIV-derived retroviral vector particle comprising the DNA sequence of interest; and
 - b) returning the cells obtained in step a) to the mammal.
48. The method of Claim 47 wherein the mammal is a human.
49. The method of Claim 47 wherein the DNA sequence of interest is a heterologous therapeutic protein.

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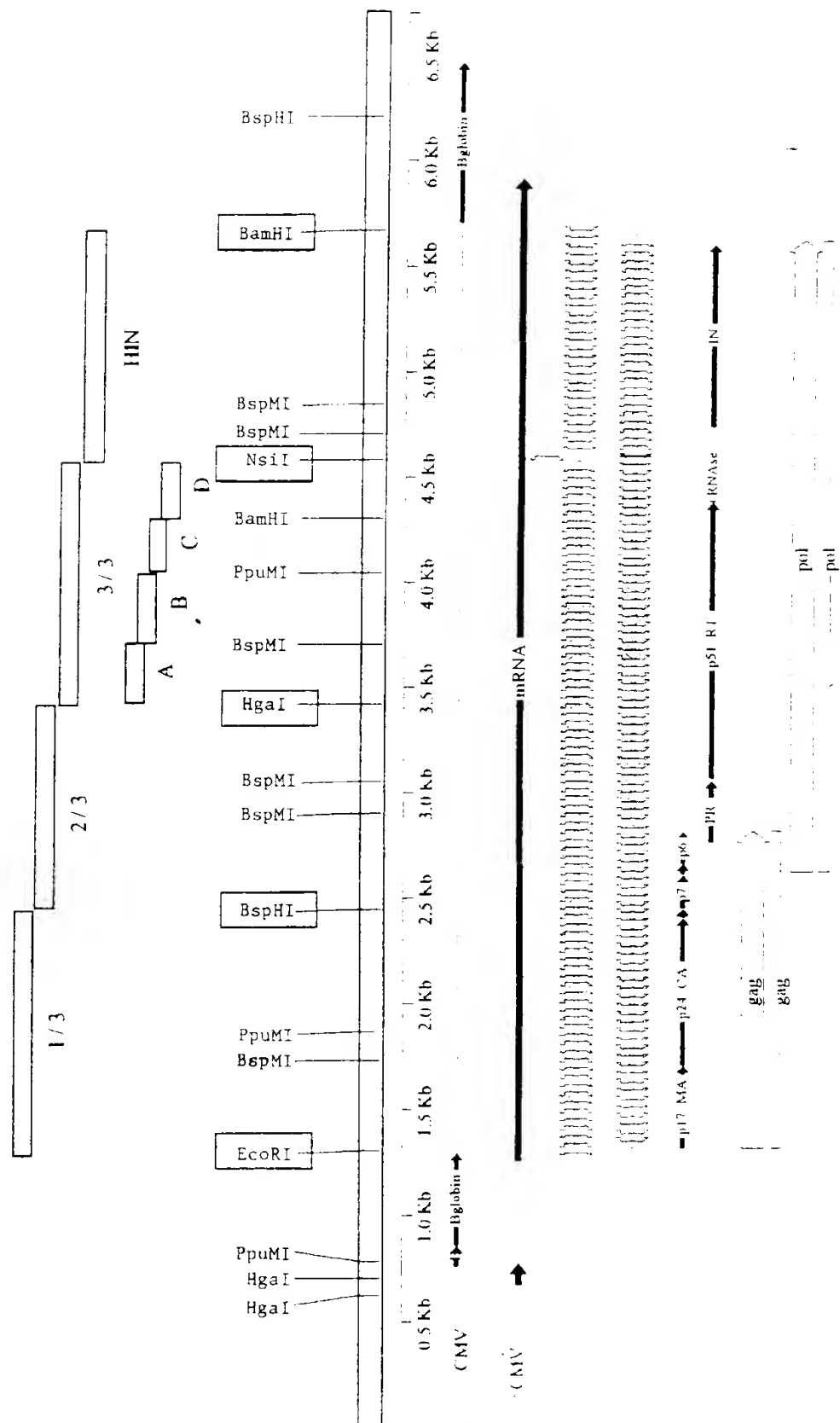


Fig. 1

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Codon Usage Frequencies

Amino Acid	pNL4-3 gagpol	mam	Amino Acid	pNL4-3 gagpol	mam	Amino Acid	pNL4-3 gagpol	mam
gca Ala(A)	58	13	gga Gly(G)	55	14	cca Pro(P)	53	16
gcc Ala(A)	23	53	ggc Gly(G)	12	50	ccc Pro(P)	17	48
gcg Ala(A)	5	17	ggg Gly(G)	27	24	ccg Pro(P)	2	17
gcu Ala(A)	14	17	ggg Gly(G)	6	12	ccu Pro(P)	27	19
aga Arg(R)	63	10	cac His(H)	24	79	age Ser(S)	29	34
agg Arg(R)	30	18	cau His(H)	76	21	agu Ser(S)	26	10
cga Arg(R)	4	6				uca Ser(S)	26	5
cgc Arg(R)	0	37	aua Ile(I)	57	5	ucc Ser(S)	7	28
cgg Arg(R)	3	21	auc Ile(I)	17	77	ucg Ser(S)	4	9
cgu Arg(R)	0	7	auu Ile(I)	26	18	ucu Ser(S)	6	13
aac Asn(N)	27	78	cua Leu(L)	15	3	aca Thr(T)	52	14
aaU Asn(N)	73	22	cuc Leu(L)	10	26	acc Thr(T)	18	57
gac Asp(D)	40	75	cug Leu(L)	11	58	acg Thr(T)	1	15
gau Asp(D)	60	25	cuu Leu(L)	11	5	acu Thr(T)	29	14
ugc Cys(C)	14	68	uua Leu(L)	40	2	ugg Trp(W)	100	100
ugu Cys(C)	26	32	uug Leu(L)	13	6			
caa Gln(Q)	56	12	aaa Lys(K)	69	18	uac Tyr(Y)	26	74
cag Gln(Q)	44	88	aag Lys(K)	31	82	uau Tyr(Y)	74	26
			aug Met(M)	100	100	gua Val(V)	58	5
gaa Glu(E)	70	25	uuc Phe(F)	40	80	guc Val(V)	13	25
gag Glu(E)	30	75	uuu Phe(F)	60	20	gug Val(V)	16	64
						guu Val(V)	14	7

Fig. 2

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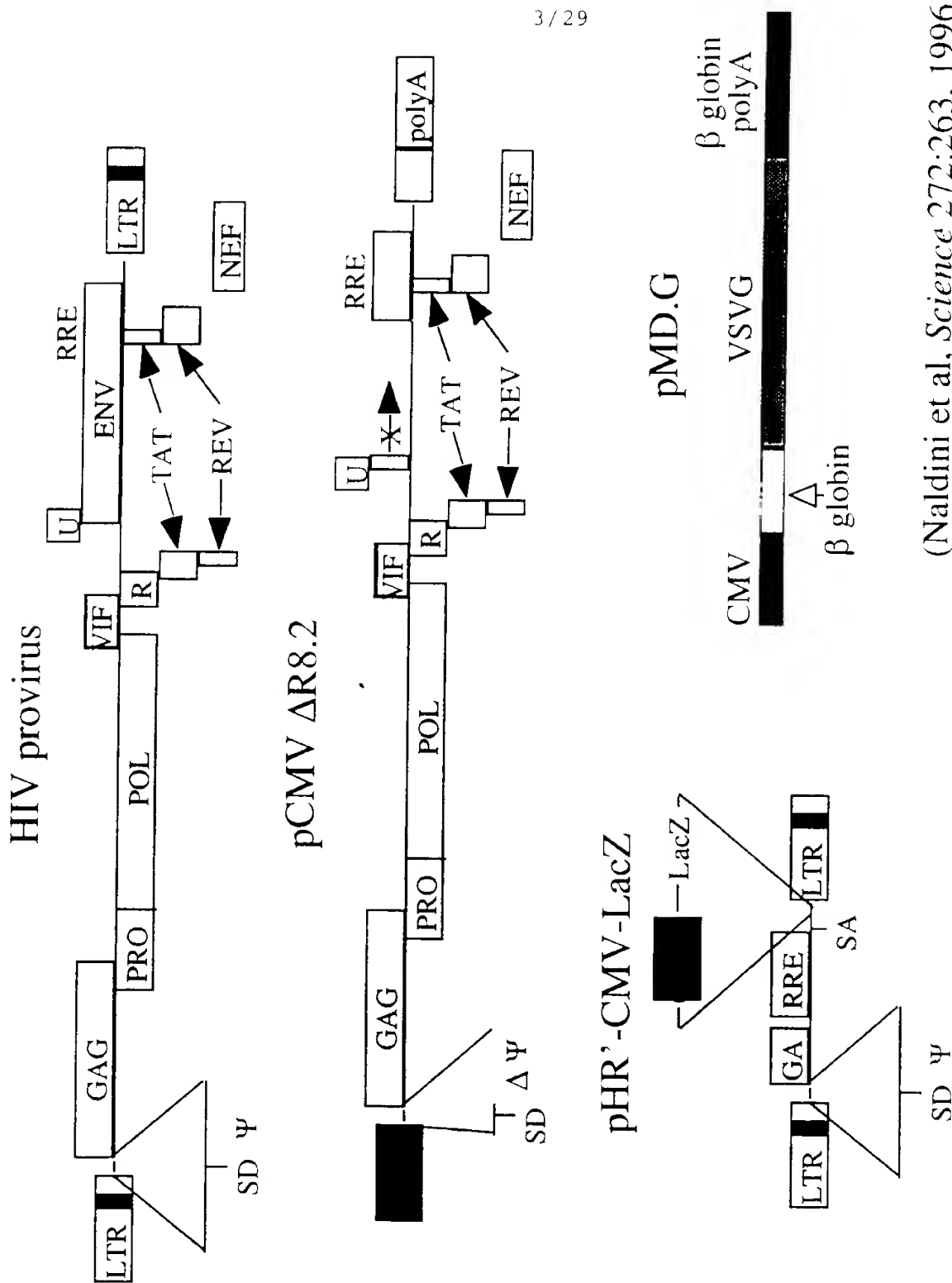


Fig. 3

(Naldini et al, *Science* 272:263, 1996)

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Rev

- **Regulates HIV gene expression by promoting cytoplasmic levels of unspliced and singly spliced mRNAs**
- **Postulated to affect splicing, stability, transport, and translation**

Fig. 4

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Codon Optimization of HIV *gagpol*

- **Remove A-rich instability elements**
- **Improve translational efficiency**
- **Reduce risk of recombination with transfer vector**

Fig. 5

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Inactivation of Inhibitory Sequences in *gag*

Schwartz, S., *et al.*

336 atg ggt gcg aga gcg tca gta tta agc ggg gga gaa tta gat cga tgg gaa aaa att cgg
396
tta agg cca ggg gga aag aaa aaa tat aaa tta aaa cat ata gta tgg gca agc agg gag
456 G G C G C G C C
cta gaa cga ttc gca gtt aat cct ggc ctg tta gaa aca tca gaa ggc tgt aga caa ata
516 M2
ctg gga cag cta caa cca tcc ctt cag aca gga tca gaa gaa ctt aga tca tta tat aat
576 M3 G G C C C C
aca gta gca acc ctc tat tgt gtg cat caa agg ata gag ata aaa gac acc aag gaa gct
C GC C C G
636 M4
tta gac aag ata gag gaa gag caa aac aaa agt aag aaa aaa gca cag caa gca gca gct
696 GTCC G G C G
gac aca gga cac agc aat cag gtc agc caa aat tac

Fig. 6

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Nucleotide Content of HIV *gagpol*

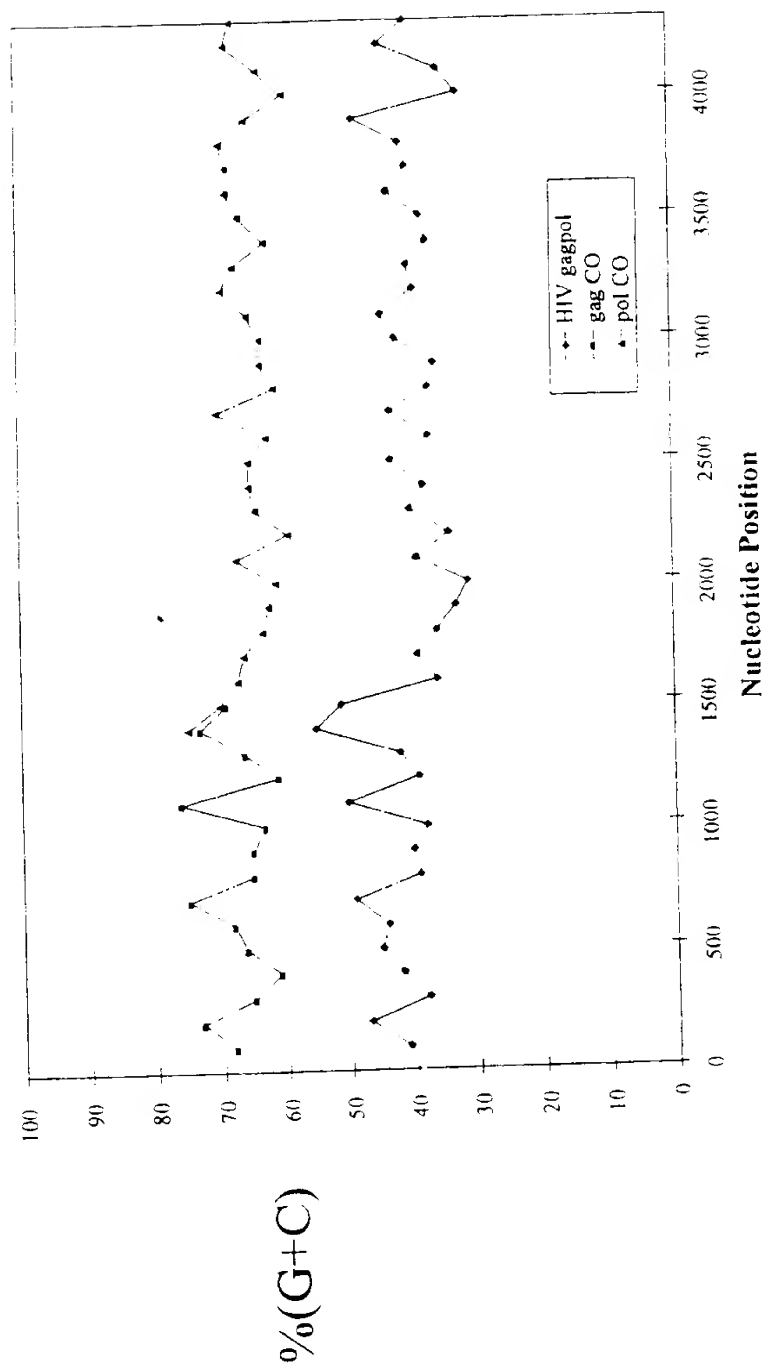


Fig. 7

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Alignment Report of Codon optimization: gag1 MEG using Clustal method with PAM250 residue weight table

810		
792	M G A P A S V L S G G E L E K	NL4-3 genbank.SEQ
792	ATG GGT GCG AGA GCG TCG GTA TTA AGC GGG GGA TAA TTA GAT AAA	
1319	M G A P A S V L S G G E L E K	pHDMHgpm2.seq
1319	ATG GGC GGC GCG GCG TCG GTG CTG TCC GGC GGC GAG CTG GAG AAG	
840		870
837	W E F I R L R P G G K K Q Y K	NL4-3 genbank.SEQ
837	TGG GAA AAA ATT CGG TTA AGG TTA GGG GGA AAG AAA CAA TAT AAA	
1364	W E F I R L R P G G K K Q Y K	pHDMHgpm2.seq
1364	TGG GAG AAG ATC CGC CTG CGC GCG GGC GGC AAG AAG CAG TAC AAG	
900		
882	L K H I V W A S R E L E R F A	NL4-3 genbank.SEQ
882	CTA AAA CAT ATA GTA TGG GCA AGC AGG GAG GTA GAA GGA TTC GCA	
1409	L K H I V W A S R E L E R F A	pHDMHgpm2.seq
1409	CTG AAG CAC ATC GTG TGG GCG TCC CGC GAG CTG GAG GCG TTC GCG	
930		960
927	V N P G L L E T S E G C R Q I	NL4-3 genbank.SEQ
927	GTT AAT CCT GGC CTT TTA GAG ACA TCA GAA GGC TGT AGA CAA ATA	
1454	V N P G L L E T S E G C R Q I	pHDMHgpm2.seq
1454	GTG AAC CCC GGC CTG CTG GAG ACC TCC GAG GGC TGC CGC CAG ATC	
990		
972	L G Q L Q P S L Q T G S E E L	NL4-3 genbank.SEQ
972	CTG GGA CAG CTA CAA CCA TCC CTT CAG ACA GGA TCA GAA GAA CTT	
1499	L G Q L Q P S L Q T G S E E L	pHDMHgpm2.seq
1499	CTG GGC CAG CTG CAG CCC TCC CTG CAA ACC GGC TCC GAG GAG CTG	
1020		1050
1017	R S L Y N T I A V L Y C V R Q	NL4-3 genbank.SEQ
1017	AGA TCA TTA TAT AAT ACA ATA GGA GTC CTC TAT TGT GTG CAT CAA	
1544	R S L Y N T I A V L Y C V R Q	pHDMHgpm2.seq
1544	CGC TCC CTG TAC AAC ACC ATC GGC GTG CTG TAC TGC GTG CAC CAG	
1080		
1062	R I D V K D T K E A L D F I E	NL4-3 genbank.SEQ
1062	AGG ATA GAT GTA AAA GAC ACC AAG GAA GCC TTA GAT AAG ATA GAG	
1589	R I D V K D T K E A L D F I E	pHDMHgpm2.seq
1589	CGC ATC GAC GTG AAG GAC ACC AAG GAG GCC CTG GAC AAG ATC GAG	
1110		1140
1107	E E Q N K S K K K A Q Q A A A	NL4-3 genbank.SEQ
1107	GAA GAG CAA AAC AAA AGT AAG AAA AAG GCA CAG CAA GCA GCA GCT	
1634	E E Q N K S K K K A Q Q A A A	pHDMHgpm2.seq
1634	GAG GAG CAG AAC AAG TCC AAG AAG AAG GCC CAG CAG GCC GCC GCC	

Fig. 8A

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Alignment Report of Codon optimization (gag).MEG. using Clustal method with PAM250 residue weight table.

1170																
1152	D	T	G	N	N	S	Q	V	S	Q	N	Y	P	I	V	NL4-3 genbank.SEQ
1152	GAC	ACA	GGA	AAC	AAC	AGC	CAG	GTG	AGC	CAA	AAT	TAC	CCT	ATA	GTG	
1673	D	T	G	N	N	S	Q	V	S	Q	N	Y	P	I	V	pHDMHgpm2.seq
1673	GAC	ACC	GGC	AAC	AAC	TCC	CAG	GTG	TCC	CAG	AAC	TAC	CCC	ATC	GTG	-
1200								1230								
1197	Q	N	L	Q	G	Q	M	V	H	Q	A	I	S	P	R	NL4-3 genbank.SEQ
1197	CAG	AAC	CTC	CAG	GGG	CAA	ATG	GTG	CAT	CAG	GCC	ATA	TCA	CCT	AGA	
1724	Q	N	L	Q	G	Q	M	V	H	Q	A	I	S	P	R	pHDMHgpm2.seq
1724	CAG	AAC	CTG	CAG	GGC	CAG	ATG	GTG	CAC	CAG	GCC	ATC	TCC	CCC	CGC	
1260																
1242	T	L	N	A	W	V	F	V	V	E	E	F	A	F	S	NL4-3 genbank.SEQ
1242	ACT	TTA	AAT	GCA	TGG	GTA	AAA	GTA	GTA	GAA	GAG	AAG	GCT	TTC	AGC	
1769	T	L	N	A	W	V	F	V	V	E	E	F	A	F	S	pHDMHgpm2.seq
1769	ACC	CTG	AAC	GCC	TGG	GTG	AAG	GTG	GTG	GAG	GAG	AAG	GCC	TTC	TCC	
1290								1320								
1287	P	E	V	I	P	M	F	S	A	L	S	F	S	A	T	NL4-3 genbank.SEQ
1287	CCA	GAA	GTA	ATA	CCC	ATG	TTT	TCA	GCA	TTA	TCA	GAA	GGA	GCC	ACC	
1814	P	E	V	I	P	M	F	S	A	L	S	F	S	A	T	pHDMHgpm2.seq
1814	CCC	GAA	GTG	ATC	CCC	ATG	TTC	TCC	GCC	CTG	TCC	GAG	GGC	GCC	ACC	
1350																
1332	P	Q	D	L	N	T	M	L	N	T	V	G	G	H	Q	NL4-3 genbank.SEQ
1332	CCA	CAA	GAT	TTA	AAT	ACC	ATG	CTA	AAC	ACA	GTG	GGG	GGA	CAT	CAA	
1859	P	Q	D	L	N	T	M	L	N	T	V	G	G	H	Q	pHDMHgpm2.seq
1859	CCC	CAG	GAC	CTG	AAC	ACC	ATG	CTG	AAC	ACC	GTG	GGC	GGC	CAC	CAG	
1380								1410								
1377	A	A	M	Q	M	L	F	E	T	I	N	E	S	A	A	NL4-3 genbank.SEQ
1377	GCA	GCC	ATG	CAA	ATG	TTA	AAA	GAG	ACC	ATC	AAT	GAG	GAA	GCT	GCA	
1904	A	A	M	Q	M	L	K	E	T	I	N	E	E	A	A	pHDMHgpm2.seq
1904	GCC	GCC	ATG	CAG	ATG	CTG	AAG	GAG	ACC	ATC	AAC	GAG	GAG	GCC	GCC	
1440																
1422	E	W	D	R	L	H	P	V	H	A	G	P	I	A	P	NL4-3 genbank.SEQ
1422	GAA	TGG	GAT	AGA	TTG	CAT	CCA	GTG	CAT	GCA	GGG	CCT	ATT	GCA	CCA	
1949	E	W	D	R	L	H	P	V	H	A	G	P	I	A	P	pHDMHgpm2.seq
1949	GAG	TGG	GAC	CGC	CTG	CAC	CCC	GTG	CAC	GCC	GGC	CCC	ATC	GCC	CCC	
1470								1500								
1467	G	Q	M	R	E	P	R	G	S	D	I	A	G	T	T	NL4-3 genbank.SEQ
1467	GGC	CAG	ATG	AGA	GAA	CCA	AGG	GGA	AGT	GAC	ATA	GCA	GGA	ACT	ACT	
1994	G	Q	M	R	E	P	R	G	S	D	I	A	G	T	T	pHDMHgpm2.seq
1994	GGC	CAG	ATG	CGC	GAG	CCC	CGC	GGC	TCC	GAC	ATC	GCC	GGC	ACC	ACC	

Fig. 8B

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Alignment Report of Codon optimization (gag) MEG using Clustal method with PAM250 residue weight table

1530																
1512	S	T	L	Q	E	Q	I	G	W	M	T	H	N	P	P	NL4-3 genbank.SEQ
1512	AGT	ACC	CTT	CAG	GAA	CAA	ATA	GGA	TGG	ATG	ACA	CAT	AAT	CCA	CGT	
2034	S	T	L	Q	E	Q	I	G	W	M	T	H	N	P	P	pHDMHgpm1.seq
2034	TCC	ACC	CTG	CAA	GAG	CAG	ATC	GGC	TGG	ATG	ACC	CAC	AAC	TCC	CCC	
1560																
1590																
1557	I	F	V	G	E	I	Y	K	R	W	I	I	L	G	L	NL4-3 genbank.SEQ
1557	ATC	CCA	GTA	GGA	GAA	ATC	TAT	AAA	AGA	TGG	ATA	ATC	CTG	GGA	TTA	
2084	I	F	V	G	E	I	Y	K	R	W	I	I	L	G	L	pHDMHgpm2.seq
2084	ATC	CCC	GTG	GGC	GAG	ATC	TAC	AAG	CCT	TGG	ATC	ATC	CTG	GGC	CTG	
1620																
1602	N	K	I	V	R	M	Y	S	P	T	S	I	L	D	I	NL4-3 genbank.SEQ
1602	AAT	AAA	ATA	GTA	AGA	ATG	TAT	AGC	CGT	ACC	AGC	ATT	CTG	GAC	ATA	
2124	N	K	I	V	R	M	Y	S	P	T	S	I	L	D	I	pHDMHgpm2.seq
2124	AAC	AAG	ATC	GTG	CGC	ATG	TAC	TCC	CTC	ACC	TCC	ATC	CTG	GAC	ATC	
1650																
1680																
1647	R	Q	G	P	K	E	P	F	R	D	Y	V	D	F	F	NL4-3 genbank.SEQ
1647	AGA	CAA	GGA	CCA	AAG	GAA	CCC	TTT	AGA	GAC	TAT	GTA	GAC	CGA	TTC	
2174	R	Q	G	P	K	E	P	F	R	D	Y	V	D	F	F	pHDMHgpm2.seq
2174	CGC	CAG	GGC	CCC	AAG	GAG	CCC	TTC	CCT	GAC	TAC	GTG	GAC	CGC	TTC	
1710																
1692	Y	K	T	L	R	A	E	Q	A	S	Q	E	V	K	N	NL4-3 genbank.SEQ
1692	TAT	AAA	ACT	CTA	AGA	GCC	GAG	CAA	GCT	TCA	CAA	GAG	GTA	AAA	AAT	
2219	Y	K	T	L	R	A	E	Q	A	S	Q	E	V	K	N	pHDMHgpm2.seq
2219	TAC	AAG	ACC	CTG	CGC	GCC	GAG	CAG	GCT	TCC	CAG	GAG	GTA	AAG	AAC	
1740																
1770																
1737	W	M	T	E	T	L	L	V	Q	N	A	N	P	I	C	NL4-3 genbank.SEQ
1737	TGG	ATG	ACA	GAA	ACC	TTG	TTG	GTC	CAA	AAT	GCG	AAC	CCA	GAT	TGT	
2264	W	M	T	E	T	L	L	V	Q	N	A	N	P	I	C	pHDMHgpm2.seq
2264	TGG	ATG	ACC	GAG	ACC	CTG	CTG	GTG	CAG	AAC	GCC	AAC	CCC	GAC	TGC	
1800																
1782	K	T	I	L	R	A	L	G	P	G	A	T	L	E	E	NL4-3 genbank.SEQ
1782	AAG	ACT	ATT	TTA	AAA	GCA	TTG	GGA	CCT	GGA	GCG	ACA	CTA	GAA	GAA	
2309	K	T	I	L	R	A	L	G	P	G	A	T	L	E	E	pHDMHgpm1.seq
2309	AAG	ACC	ATC	CTG	AAG	GCC	CTG	GGC	CTC	GGC	GCC	ACC	CTG	GAG	GAG	
1830																
1860																
1827	M	M	T	A	C	Q	G	V	S	G	P	G	H	F	A	NL4-3 genbank.SEQ
1827	ATG	ATG	ACA	GCA	TGT	CAG	GGA	GTG	GCG	GGA	CCC	GGC	CAT	AAA	GCA	
2354	M	M	T	A	C	Q	G	V	S	G	P	G	H	F	A	pHDMHgpm2.seq
2354	ATG	ATG	ACC	GCC	TGC	CAG	GGC	GTG	GCT	GGC	CCC	GGC	CAC	AAG	GCC	

Fig. 8C

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Alignment Report of Codon optimization (gag).MEG, using Clustal method with PAM250 residue weight table

1890																
1872	R	V	L	A	E	A	M	S	Q	V	T	N	P	A	T	NL4-3 genbank.SEQ
1872	AGA	GTT	TTG	GCT	GAA	GCA	ATG	AGC	CPA	GTA	ACA	AAT	CCA	GCT	ACC	
2399	R	V	L	A	E	A	M	S	Q	V	T	N	P	A	T	pHDMHgpm2.seq
2399	CAC	GTG	CTG	GCC	GAG	GCC	ATG	TCC	CAA	GTC	ADC	AAC	CCC	GCC	ACC	
1920																
1917	I	M	I	Q	K	G	N	F	R	N	Q	R	K	T	V	NL4-3 genbank.SEQ
1917	ATA	ATG	ATA	CAG	AAA	GGC	AAT	TTT	AGG	AAC	CAA	AGA	AAG	ACT	GTT	
2444	I	M	I	Q	K	G	N	F	R	N	Q	R	K	T	V	pHDMHgpm2.seq
2444	ATC	ATG	ATC	CAG	AAG	GGC	AAC	TTC	CSC	AAC	CAG	CSC	AAG	ACC	GTG	
1980																
1962	F	C	F	N	C	G	K	E	G	H	I	A	K	N	C	NL4-3 genbank.SEQ
1962	AAG	TGT	TTC	AAT	TGT	GGC	AAA	GAA	GGG	CAC	ATA	GCC	AAA	AAT	TGC	
2489	F	C	F	N	C	G	K	E	G	H	I	A	K	N	C	pHDMHgpm2.seq
2489	AAG	TGC	TTC	AAC	TGC	GGC	AAG	GAG	GGC	CAC	ATC	GTC	AAG	AAC	TGC	
2010																
2007	F	A	P	R	K	K	G	C	W	K	C	G	F	E	G	NL4-3 genbank.SEQ
2007	AGG	GCC	CCT	AGG	AAA	AAG	GGC	TGT	TGG	AAA	TGT	GGA	AAG	GAA	GGA	
2534	P	A	P	R	K	K	G	C	W	K	C	G	F	E	G	pHDMHgpm2.seq
2534	CGC	GCC	CCC	CGC	AAG	AAG	GGC	TGC	TGG	AAG	TGC	GGC	AAG	GAG	GGC	
2070																
2052	H	Q	M	K	D	C	T	E	R	Q	A	N	F	L	G	NL4-3 genbank.SEQ
2052	CAC	CAA	ATG	AAA	GAT	TGT	ACT	GAG	AGA	CAG	GCT	AAT	TTT	TTA	GGG	
2579	H	Q	M	K	D	C	T	E	R	Q	A	N	F	L	G	pHDMHgpm2.seq
2579	CAC	CAG	ATG	AAA	GAT	TGT	ACT	GAG	AGA	CAG	GCT	AAT	TTT	TTA	GGG	
2100																
2097	K	I	W	P	S	H	K	G	R	P	G	N	F	L	Q	NL4-3 genbank.SEQ
2097	AAG	ATC	TGG	CCT	TCC	CAC	AAG	GGA	AGG	CCA	GGG	AAT	TTT	CTT	CAG	
2624	K	I	W	P	S	H	K	G	R	P	G	N	F	L	Q	pHDMHgpm2.seq
2624	AAG	ATC	TGG	CCT	TCC	CAC	AAG	GGA	AGG	CCA	GGG	AAT	TTT	CTT	CAG	
2160																
2142	S	R	P	E	P	T	A	P	P	E	E	S	F	R	F	NL4-3 genbank SEQ
2142	AGT	AGA	CCA	GAG	CCA	ACA	GCC	CCA	CCA	GAA	GAG	AGT	TTT	AGG	TTT	
2669	S	R	P	E	P	T	A	P	P	E	E	S	F	R	F	pHDMHgpm2.seq
2669	AGT	AGA	CCA	GAG	CCA	ACA	GCC	CCA	CCA	GAA	GAG	AGT	TTT	AGG	TTT	
2190																
2187	G	E	E	T	T	T	P	S	Q	K	Q	E	P	I	D	NL4-3 genbank.SEQ
2187	GGG	GAA	GAG	ACA	ACA	ACT	CCC	TCT	CAG	AAG	CAG	GAG	CCG	ATA	GAC	
2714	G	E	E	T	T	T	P	S	Q	K	Q	E	P	I	D	pHDMHgpm2.seq
2714	GGG	GAA	GAG	ACA	ACA	ACT	CCC	TCT	CAG	AAG	CAG	GAG	CCG	ATA	GAC	

Fig. 8D

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Alignment Report of Codon optimization (gag).MEG using Clustal method with PAM250 residue weight table

2250																
2232	K	E	L	Y	P	L	A	S	L	R	S	L	F	S	NL4-3 genbank.SEQ	
2232	AAG	GAA	CTG	TAT	CCT	TTA	GCT	TCC	CTC	AGA	TCA	CTC	TTT	GGC	AGC	
2759	K	E	L	Y	P	L	A	S	L	R	S	L	F	G	S	pHDMHgpm2.seq
2759	AAG	GAA	CTG	TAT	CCT	TTA	GCT	TCC	CTC	AGA	TCA	CTC	TTT	GGC	AGC	
2280																
2277	D	P	S	S	Q	NL4-3 genbank.SEQ
2277	GAC	CCC	TCG	TCA	CAA	TAA	
2804	D	P	S	S	Q	pHDMHgpm2.seq
2804	GAC	CCC	TCG	TCA	CAA	TAA	

Fig. 8E

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Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table

2090															2120															
2087	F	F	R	E	D	L	A	F	P	Q	G	K	A	R	E	NL4-3 genbank.SEQ														
2087	TTT	TTT	AGG	GAA	GAT	CTG	GCC	TTC	CCA	CAA	GGG	AAG	GCC	AGG	GAA	-														
2085	F	F	R	E	D	L	A	F	P	Q	G	K	A	R	E	pNL4-3.seq														
2085	TTT	TTT	AGG	GAA	GAT	CTG	GCC	TTC	CCA	CAA	GGG	AAG	GCC	AGG	GAA															
2612	F	F	R	E	D	L	A	F	P	Q	G	K	A	R	E	pHDMHgpm2.seq														
2612	TTT	TTT	AGG	GAA	GAT	CTG	GCC	TTC	CCA	CAA	GGG	AAG	GCC	AGG	GAA															
2150																														
2132	F	S	S	E	Q	T	R	A	N	S	P	T	R	R	E	NL4-3 genbank.SEQ														
2132	TTT	TCT	TCA	GAG	CAG	ACC	AGA	GCC	AAC	AGC	CCC	ACC	AGA	AGA	GAG															
2130	F	S	S	E	Q	T	R	A	N	S	P	T	R	R	E	pNL4-3.seq														
2130	TTT	TCT	TCA	GAG	CAG	ACC	AGA	GCC	AAC	AGC	CCC	ACC	AGA	AGA	GAG															
2657	F	S	S	E	Q	T	R	A	N	S	P	T	R	R	E	pHDMHgpm2.seq														
2657	TTT	TCT	TCA	GAG	CAG	ACC	AGA	GCC	AAC	AGC	CCC	ACC	AGA	AGA	GAG															
2180															2210															
2177	L	Q	V	W	G	R	D	N	N	S	L	S	E	A	G	NL4-3 genbank.SEQ														
2177	CTT	CAG	GTT	TGG	GGA	AGA	GAC	AAC	AAC	TCC	CTC	TCA	GAA	GCA	GGA															
2175	L	Q	V	W	G	R	D	N	N	S	L	S	E	A	G	pNL4-3.seq														
2175	CTT	CAG	GTT	TGG	GGA	AGA	GAC	AAC	AAC	TCC	CTC	TCA	GAA	GCA	GGA															
2702	L	Q	V	W	G	R	D	N	N	S	L	S	E	A	G	pHDMHgpm2.seq														
2702	CTT	CAG	GTT	TGG	GGA	AGA	GAC	AAC	AAC	TCC	CTC	TCA	GAA	GCA	GGA															
2240																														
2222	A	D	R	Q	G	T	V	S	F	S	F	P	Q	I	T	NL4-3 genbank.SEQ														
2222	GCC	GAT	AGA	CAA	GGA	ACT	GTA	TCC	TTT	AGC	TTC	CCT	CAG	ATC	ACT															
2220	A	D	R	Q	G	T	V	S	F	S	F	P	Q	I	T	pNL4-3.seq														
2220	GCC	GAT	AGA	CAA	GGA	ACT	GTA	TCC	TTT	AGC	TTC	CCT	CAG	ATC	ACT															
2747	A	D	R	Q	G	T	V	S	F	S	F	P	Q	I	T	pHDMHgpm2.seq														
2747	GCC	GAT	AGA	CAA	GGA	ACT	GTA	TCC	TTT	AGC	TTC	CCT	CAG	ATC	ACT															
2270															2300															
2267	L	W	Q	R	P	L	V	T	I	K	I	G	G	Q	L	NL4-3 genbank.SEQ														
2267	CTT	TGG	CAG	CGA	CCC	CTC	GTC	ACA	ATA	AAG	ATA	GAG	GAG	CAA	TTA															
2265	L	W	Q	R	P	L	V	T	I	K	I	G	G	Q	L	pNL4-3.seq														
2265	CTT	TGG	CAG	CGA	CCC	CTC	GTC	ACA	ATA	AAG	ATA	GAG	GAG	CAA	TTA															
2792	L	W	Q	R	P	L	V	T	I	K	I	G	G	Q	L	pHDMHgpm2.seq														
2792	CTT	TGG	CAG	CGA	CCC	CTC	GTC	ACA	ATA	AAG	ATA	GAG	GAG	CAA	TTA															
2330																														
2312	K	E	A	L	L	D	T	G	A	D	D	T	V	L	E	NL4-3 genbank.SEQ														
2312	AAG	GAA	GCT	CTA	TTA	GAT	ACA	GGA	GCA	GAT	GAT	ACA	GTA	TTA	GAA															
2310	K	E	A	L	L	D	T	G	A	D	D	T	V	L	E	pNL4-3.seq														
2310	AAG	GAA	GCT	CTA	TTA	GAT	ACA	GGA	GCA	GAT	GAT	ACA	GTA	TTA	GAA															
2837	K	E	A	L	L	D	T	G	A	D	D	T	V	L	E	pHDMHgpm2.seq														
2837	AAG	GAG	GCC	CTG	CTG	GAC	ACC	GGC	GCC	GAC	GAC	ACC	GTG	CTG	GAG															

Fig. 9A

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Alignment Report of Codon Optimization (pol) MEG, using Clustal method with PAM250 residue weight table.

2360															2380														
2357	E	M	N	L	P	G	R	W	K	P	K	M	I	G	G	NL4-3 genbank.SEQ													
2357	GAA	ATG	AAT	TTG	CCA	GGA	AGA	TGG	AAA	CCA	AAA	ATG	ATA	GGG	GGA														
2355	E	M	N	L	P	G	R	W	K	P	K	M	I	G	G	pNL4-3.seq													
2355	GAA	ATG	AAT	TTG	CCA	GGA	AGA	TGG	AAA	CCA	AAA	ATG	ATA	GGG	GGA														
2382	E	M	N	L	P	G	R	W	K	P	K	M	I	G	G	pHDMHqpm1.seq													
2382	GAG	ATG	AAC	CTG	CCC	GGC	CGC	TGG	AAG	CCC	AAG	ATG	ATC	GGC	GGC														
2420																													
2402	I	G	G	F	I	K	V	G	Q	Y	D	Q	I	L	I	NL4-3 genbank.SEQ													
2402	ATT	GGA	GST	TTT	ATC	AAA	GTA	GGA	CAG	TAT	GAT	CAG	ATA	CTC	ATA														
2400	I	G	G	F	I	K	V	R	Q	Y	D	Q	I	L	I	pNL4-3.seq													
2400	ATT	GGA	GGT	TTT	ATC	AAA	GTA	AGA	CAG	TAT	GAT	CAG	ATA	CTC	ATA														
2927	I	G	G	F	I	K	V	R	Q	Y	D	Q	I	L	I	pHDMHqpm2.seq													
2927	ATC	GGC	GGC	TTG	ATC	AAA	GTC	CGC	CAG	TAC	GAC	CAG	ATC	CTG	ATC														
2450															2480														
2447	E	I	C	G	H	K	A	I	G	T	V	L	V	G	P	NL4-3 genbank.SEQ													
2447	GAA	ATC	TGC	GGA	CAT	AAA	GCT	ATA	GGT	ACA	GTA	TTA	GTA	GGA	CCT														
2445	E	I	C	G	H	K	A	I	G	T	V	L	V	G	P	pNL4-3.seq													
2445	GAA	ATC	TGC	GGA	CAT	AAA	GCT	ATA	GGT	ACA	GTA	TTA	GTA	GGA	CCT														
2972	E	I	C	G	H	K	A	I	G	T	V	L	V	G	P	pHDMHqpm2.seq													
2972	GAG	ATC	TGC	GGC	CAC	AAG	GCC	ATC	GGC	ACC	GTG	CTG	GTG	GGC	CCC														
2510																													
2492	T	P	V	N	I	I	G	R	N	L	L	T	Q	I	G	NL4-3 genbank.SEQ													
2492	ACA	CCT	GTC	AAC	ATA	ATT	GGA	AGA	AAT	CTG	TTG	ACT	CAG	ATT	GGC														
2490	T	P	V	N	I	I	G	R	N	L	L	T	Q	I	G	pNL4-3.seq													
2490	ACA	CCT	GTC	AAC	ATA	ATT	GGA	AGA	AAT	CTG	TTG	ACT	CAG	ATT	GGC														
3017	T	P	V	N	I	I	G	R	N	L	L	T	Q	I	G	pHDMHqpm2.seq													
3017	ACC	CCC	GTG	AAC	ATC	ATC	GGC	CGC	AAC	CTG	CTG	ACC	CAG	ATC	GGC														
2540															2570														
2537	C	T	L	N	F	P	I	S	P	I	E	T	V	P	V	NL4-3 genbank.SEQ													
2537	TGC	ACT	TTA	AAT	TTT	CCC	ATT	AGT	CCT	ATT	GAG	ACT	GTA	CCA	GTA														
2535	C	T	L	N	F	P	I	S	P	I	E	T	V	P	V	pNL4-3.seq													
2535	TGC	ACT	TTA	AAT	TTT	CCC	ATT	AGT	CCT	ATT	GAG	ACT	GTA	CCA	GTA														
3062	C	T	L	N	F	P	I	S	P	I	E	T	V	P	V	pHDMHqpm2.seq													
3062	TGC	ACC	CTG	AAC	TTG	CCC	ATC	TCC	CCC	ATC	GAG	ACC	GTG	CCC	GTG														
2600																													
2582	K	L	K	P	G	M	D	G	P	K	V	K	Q	W	P	NL4-3 genbank.SEQ													
2582	AAA	TTA	AAG	CCA	GGA	ATG	GAT	GGC	CCA	AAA	GTT	AAA	CAA	TGG	CCA														
2580	K	L	K	P	G	M	D	G	P	K	V	K	Q	W	P	pNL4-3.seq													
2580	AAA	TTA	AAG	CCA	GGA	ATG	GAT	GGC	CCA	AAA	GTT	AAA	CAA	TGG	CCA														
3107	K	L	K	P	G	M	D	G	P	K	V	K	Q	W	P	pHDMHqpm2.seq													
3107	AAG	CTG	AAG	CCC	GGC	ATG	GAC	GGC	CCC	AAA	GTC	AAG	CAG	TGG	CCC														

Fig. 9B

15/29

Alignment Report of Codon Optimization (pol) MEG using Clustal method with PAM250 residue weight table

2630															2660															
2627	L	T	E	E	K	I	K	A	L	V	E	I	C	T	E	NL4-3 genbank.SEQ														
2627	TTG	ACA	GAA	GAA	AAA	ATA	AAA	GCA	TTA	GTA	GAA	ATT	TGT	ACA	GAA															
2625	L	T	E	E	K	I	K	A	L	V	E	I	C	T	E	pNL4-3.seq														
2625	TTG	ACA	GAA	GAA	AAA	ATA	AAA	GCA	TTA	GTA	GAA	ATT	TGT	ACA	GAA															
3152	L	T	E	E	K	I	K	A	L	V	E	I	C	T	E	pHDMHqpm2.seq														
3152	CTG	ACC	GAG	GAG	AAG	ATC	AAG	GCC	CTG	GTG	GAG	ATC	TGC	ACC	GAG															
2690																														
2672	M	E	K	E	G	K	I	S	K	I	G	P	E	N	P	NL4-3 genbank.SEQ														
2672	ATG	GAA	AAG	GAA	GGA	AAA	ATT	TCA	AAA	ATT	GGG	CCT	GAA	AAT	CCA															
2670	M	E	K	E	G	K	I	S	K	I	G	P	E	N	P	pNL4-3.seq														
2670	ATG	GAA	AAG	GAA	GGA	AAA	ATT	TCA	AAA	ATT	GGG	CCT	GAA	AAT	CCA															
3197	M	E	K	E	G	K	I	S	K	I	G	P	E	N	P	pHDMHqpm2.seq														
3197	ATG	GAG	AAG	GAG	GGC	AAG	ATC	TCC	AAG	ATC	GGC	CCC	GAG	AAC	CCC															
2720															2750															
2717	Y	N	T	P	V	F	A	I	K	K	K	D	S	T	K	NL4-3 genbank.SEQ														
2717	TAC	AAT	ACT	CCA	GTA	TTT	GCC	ATA	AAG	AAA	AAA	GAC	AGT	ACT	AAA															
2715	Y	N	T	P	V	F	A	I	K	K	K	D	S	T	K	pNL4-3.seq														
2715	TAC	AAT	ACT	CCA	GTA	TTT	GCC	ATA	AAG	AAA	AAA	GAC	AGT	ACT	AAA															
3242	Y	N	T	P	V	F	A	I	K	K	K	D	S	T	K	pHDMHqpm2.seq														
3242	TAC	AAC	ACC	CCC	GTG	TTC	GCC	ATC	AAG	AAG	AAG	GAC	TCC	ACC	AAG															
2780																														
2762	W	R	K	L	V	D	F	R	E	L	N	K	R	T	Q	NL4-3 genbank.SEQ														
2762	TGG	AGA	AAA	TTA	GTA	GAT	TTC	AGA	GAA	CTT	AAT	AAG	AGA	ACT	CAA															
2760	W	R	K	L	V	D	F	R	E	L	N	K	R	T	Q	pNL4-3.seq														
2760	TGG	AGA	AAA	TTA	GTA	GAT	TTC	AGA	GAA	CTT	AAT	AAG	AGA	ACT	CAA															
3287	W	R	K	L	V	D	F	R	E	L	N	K	R	T	Q	pHDMHqpm2.seq														
3297	TGG	CGC	AAG	CTG	GTG	GAC	TTC	CGC	GAG	CTG	AAC	AAG	CGC	ACC	CAG															
2810															2840															
2807	D	F	W	E	V	Q	L	G	I	P	H	P	A	G	L	NL4-3 genbank.SEQ														
2807	GAT	TTC	TGG	GAA	GTT	CAA	TTA	GGA	ATA	CCA	CAT	CCT	GCA	GGG	TTA															
2805	D	F	W	E	V	Q	L	G	I	P	H	P	A	G	L	pNL4-3.seq														
2805	GAT	TTC	TGG	GAA	GTT	CAA	TTA	GGA	ATA	CCA	CAT	CCT	GCA	GGG	TTA															
3332	D	F	W	E	V	Q	L	G	I	P	H	P	A	G	L	pHDMHqpm2.seq														
3332	GAC	TTC	TGG	GAG	GTG	CAG	CTG	GGC	ATC	CCC	CAC	CCC	GCC	GGC	CTG															
2870																														
2852	K	Q	K	K	S	V	T	V	L	D	V	G	D	A	Y	NL4-3 genbank.SEQ														
2852	AAA	CAG	AAA	AAA	TCA	GTA	ACA	GTA	CTG	GAT	GTG	GGC	GAT	GCA	TAT															
2850	K	Q	K	K	S	V	T	V	L	D	V	G	D	A	Y	pNL4-3.seq														
2850	AAA	CAG	AAA	AAA	TCA	GTA	ACA	GTA	CTG	GAT	GTG	GGC	GAT	GCA	TAT															
3377	K	Q	K	K	S	V	T	V	L	D	V	G	D	A	Y	pHDMHqpm2.seq														
3377	AAG	CAG	AAG	AAG	TCC	GTG	ACC	GTG	CTG	GAC	GTG	GGC	GAC	GCC	TAC															

Fig. 9C

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Alignment Report of Codon Optimization (poi) MEG, using Clustal method with PAM250 residue weight table

2900															2930														
2897	F	S	V	P	L	D	K	D	F	R	K	Y	T	A	F	NL4-3 genbank.SEQ													
2897	TTT	TCA	GTT	CCC	TTA	GAT	AAA	GAC	TTC	AGG	AAG	TAT	ACT	GCA	TTT														
2895	F	S	V	P	L	D	K	D	F	R	K	Y	T	A	F	pNL4-3.seq													
2895	TTT	TCA	GTT	CCC	TTA	GAT	AAA	GAC	TTC	AGG	AAG	TAT	ACT	GCA	TTT														
3422	F	S	V	P	L	D	K	D	F	R	K	Y	T	A	F	pHDMHgpm2.seq													
3422	TTT	TCC	GTG	CCC	CTG	GAC	AAG	GAC	TTC	CGC	AAG	TAC	ACC	GCC	TTC														
2960																													
2942	T	I	P	S	I	N	N	E	T	P	G	I	R	Y	Q	NL4-3 genbank.SEQ													
2942	ACC	ATA	CCT	AGT	ATA	AAC	AAT	GAG	ACA	CCA	GGG	ATT	AGA	TAT	CAG														
2940	T	I	P	S	I	N	N	E	T	P	G	I	R	Y	Q	pNL4-3.seq													
2940	ACC	ATA	CCT	AGT	ATA	AAC	AAT	GAG	ACA	CCA	GGG	ATT	AGA	TAT	CAG														
3467	T	I	P	S	I	N	N	E	T	P	G	I	R	Y	Q	pHDMHgpm2.seq													
3467	ACC	ATC	CCC	TCC	ATC	AAC	AAC	GAG	ACC	CCC	GGC	ATC	CGC	TAC	CAG														
2990															3020														
2987	Y	N	V	L	P	Q	G	W	K	G	S	P	A	I	F	NL4-3 genbank.SEQ													
2987	TAC	AAT	GTG	CTT	CCA	CAG	GGA	TGG	AAA	GGA	TCA	CCA	GCA	ATA	TTC														
2985	Y	N	V	L	P	Q	G	W	K	G	S	P	A	I	F	pNL4-3.seq													
2985	TAC	AAT	GTG	CTT	CCA	CAG	GGA	TGG	AAA	GGA	TCA	CCA	GCA	ATA	TTC														
3512	Y	N	V	L	P	Q	G	W	K	G	S	P	A	I	F	pHDMHgpm2.seq													
3512	TAC	AAC	GTG	CTG	CCC	CAG	GGC	TGG	AAG	GTC	TCC	CCC	GCC	ATC	TTC														
3050																													
3032	Q	C	S	M	T	K	I	L	E	P	F	R	K	Q	N	NL4-3 genbank.SEQ													
3032	CAG	TGT	AGC	ATG	ACA	AAA	ATC	TTA	GAG	CCT	TTT	AGA	AAA	CAA	AAT														
3030	Q	C	S	M	T	K	I	L	E	P	F	R	K	Q	N	pNL4-3.seq													
3030	CAG	TGT	AGC	ATG	ACA	AAA	ATC	TTA	GAG	CCT	TTT	AGA	AAA	CAA	AAT														
3557	Q	C	S	M	T	K	I	L	E	P	F	R	K	Q	N	pHDMHgpm2.seq													
3557	CAG	TGC	TCC	ATG	ACC	AAG	ATC	CTG	GAG	CCC	TTC	CGC	AAG	CAG	AAC														
3080															3110														
3077	P	D	I	V	I	Y	Q	Y	M	D	D	L	Y	V	G	NL4-3 genbank.SEQ													
3077	CCA	GAC	ATA	GTC	ATC	TAT	CAA	TAC	ATG	GAT	GAT	TTG	TAT	GTA	GGA														
3075	P	D	I	V	I	Y	Q	Y	M	D	D	L	Y	V	G	pNL4-3.seq													
3075	CCA	GAC	ATA	GTC	ATC	TAT	CAA	TAC	ATG	GAT	GAT	TTG	TAT	GTA	GGA														
3602	P	D	I	V	I	Y	Q	Y	M	D	D	L	Y	V	G	pHDMHgpm2.seq													
3602	CCC	GAC	ATC	GTG	ATC	TAC	CAG	TAC	ATG	GAC	GAC	CTG	TAC	GTG	GGC														
3140																													
3122	S	D	L	E	I	G	Q	H	R	T	K	I	E	E	L	NL4-3 genbank.SEQ													
3122	TCT	GAC	TTA	GAA	ATA	GGG	CAG	CAT	AGA	ACA	AAA	ATA	GAG	GAA	CTG														
3120	S	D	L	E	I	G	Q	H	R	T	K	I	E	E	L	pNL4-3.seq													
3120	TCT	GAC	TTA	GAA	ATA	GGG	CAG	CAT	AGA	ACA	AAA	ATA	GAG	GAA	CTG														
3647	S	D	L	E	I	G	Q	H	R	T	K	I	E	E	L	pHDMHgpm2.seq													
3647	TCC	GAC	CTG	GAG	ATC	GGC	CAG	CAC	CGC	ACC	AAG	ATC	GAG	GAG	CTG														

Fig. 9D

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Alignment Report of Codon Optimization (pol).MEG using Clustal method with PAM250 residue weight table

3170															3200															
3167	R	Q	H	L	L	R	W	G	F	T	T	P	D	K	K	NL4-3 genbank.SEQ														
3167	AGA	CAA	CAT	CTG	TTG	AGG	TGG	GGA	TTT	ACC	ACA	CCA	GAC	AAA	AAA															
3165	R	Q	H	L	L	R	W	G	F	T	T	P	D	K	K	pNL4-3.seq														
3165	AGA	CAA	CAT	CTG	TTG	AGG	TGG	GGA	TTT	ACC	ACA	CCA	GAC	AAA	AAA															
3692	R	Q	H	L	L	R	W	G	F	T	T	P	D	K	K	pHDMHgpm2.seq														
3692	CGC	CAG	CAC	CTG	CTG	CGC	TGG	GGC	TTC	ACC	ACC	CCC	GAC	AAG	AAG															
3230																														
3212	H	Q	K	E	P	P	F	L	W	M	G	Y	E	L	H	NL4-3 genbank.SEQ														
3212	CAT	CAG	AAA	GAA	CCT	CCA	TTC	CTT	TGG	ATG	GGT	TAT	GAA	CTC	CAT															
3210	H	Q	K	E	P	P	F	L	W	M	G	Y	E	L	H	pNL4-3.seq														
3210	CAT	CAG	AAA	GAA	CCT	CCA	TTC	CTT	TGG	ATG	GGT	TAT	GAA	CTC	CAT															
3737	H	Q	K	E	P	P	F	L	W	M	G	Y	E	L	H	pHDMHgpm2.seq														
3737	CAC	CAG	AAG	GAG	CCC	CCC	TTC	CTG	TGG	ATG	GGC	TAC	GAG	CTG	CAC															
3260															3290															
3257	P	D	K	W	T	V	Q	P	I	V	L	P	E	K	D	NL4-3 genbank.SEQ														
3257	CCT	GAT	AAA	TGG	ACA	GTA	CAG	CCT	ATA	GTG	CTG	CCA	GAA	AAG	GAC															
3255	P	D	K	W	T	V	Q	P	I	V	L	P	E	K	D	pNL4-3.seq														
3255	CCT	GAT	AAA	TGG	ACA	GTA	CAG	CCT	ATA	GTG	CTG	CCA	GAA	AAG	GAC															
3792	P	D	K	W	T	V	Q	P	I	V	L	P	E	K	D	pHDMHgpm2.seq														
3792	CCC	GAC	AAG	TGG	ACC	GTG	CAG	CCC	ATC	GTG	CTG	CCC	GAG	AAG	GAC															
3320																														
3302	S	W	T	V	N	D	I	Q	K	L	V	G	K	L	N	NL4-3 genbank.SEQ														
3302	AGC	TGG	ACT	GTC	AAT	GAC	ATA	CAG	AAA	TTA	GTG	GGA	AAA	TTG	AAT															
3300	S	W	T	V	N	D	I	Q	K	L	V	G	K	L	N	pNL4-3.seq														
3300	AGC	TGG	ACT	GTC	AAT	GAC	ATA	CAG	AAA	TTA	GTG	GGA	AAA	TTG	AAT															
3827	S	W	T	V	N	D	I	Q	K	L	V	G	K	L	N	pHDMHgpm2.seq														
3827	TCC	TGG	ACC	GTG	AAC	GAC	ATC	CAG	AAG	CTG	GTG	GGC	AAG	CTG	AAC															
3350															3380															
3347	W	A	S	Q	I	Y	A	G	I	K	V	R	Q	L	C	NL4-3 genbank.SEQ														
3347	TGG	GCA	AGT	CAG	ATT	TAT	GCA	GGG	ATT	AAA	GTA	AGG	CAA	TTA	TGT															
3345	W	A	S	Q	I	Y	A	G	I	K	V	R	Q	L	C	pNL4-3.seq														
3345	TGG	GCA	AGT	CAG	ATT	TAT	GCA	GGG	ATT	AAA	GTA	AGG	CAA	TTA	TGT															
3872	W	A	S	Q	I	Y	A	G	I	K	V	P	Q	L	C	pHDMHgpm2.seq														
3872	TGG	GCC	TCC	CAG	ATC	TAC	GCC	GGC	ATC	AAA	GTG	GGC	CAG	CTG	TGC															
3410																														
3391	K	L	L	R	G	T	K	A	L	T	E	V	V	P	L	NL4-3 genbank.SEQ														
3392	AAA	CTT	CTT	AGG	GGA	ACC	AAA	GCA	CTA	ACA	GAA	GTA	GTA	CCA	CTA															
3390	K	L	L	R	G	T	K	A	L	T	E	V	V	P	L	pNL4-3.seq														
3390	AAA	CTT	CTT	AGG	GGA	ACC	AAA	GCA	CTA	ACA	GAA	GTA	GTA	CCA	CTA															
3917	K	L	L	R	G	T	K	A	L	T	E	V	V	P	L	pHDMHgpm2.seq														
3917	AAG	CTG	CTG	CGC	GGC	ACC	AAG	GCC	CTG	ACC	GAG	GTG	GTG	CCC	CTG															

Fig. 9E

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Alignment Report of Codon Optimization: polB MEG using Clustal method with PAM250 residue weight table

3440															3470															
3437	T	E	E	A	E	L	E	L	A	E	N	R	E	I	L	NL4-3 genbank.SEQ														
3437	ACA	GAA	GAA	GCA	GAG	CTA	GAA	CTG	GCA	GAA	AAC	AGG	GAG	ACT	CTA															
3435	T	E	E	A	E	L	E	L	A	E	N	R	E	I	L	pNL4-3.seq														
3435	ACA	GAA	GAA	GCA	GAG	CTA	GAA	CTG	GCA	GAA	AAC	AGG	GAG	ATT	CTA															
3962	T	E	E	A	E	L	E	L	A	E	N	R	E	I	L	pHDMHgpm2.seq														
3962	ACC	GAG	GAG	GCC	GAG	CTG	GAG	CTG	GCC	GAG	AAC	CGC	GAG	ATC	CTG															
3500																														
3482	K	E	P	V	H	G	V	Y	Y	D	P	S	K	D	L	NL4-3 genbank.SEQ														
3482	AAA	GAA	CCG	GTA	CAT	GGA	GTG	TAT	TAT	GAC	CCA	TCA	AAA	GAC	TTA															
3480	K	E	P	V	H	G	V	Y	Y	D	P	S	K	D	L	pNL4-3.seq														
3480	AAA	GAA	CCG	GTA	CAT	GGA	GTG	TAT	TAT	GAC	CCA	TCA	AAA	GAC	TTA															
4007	K	E	P	V	H	G	V	Y	Y	D	P	S	K	D	L	pHDMHgpm2.seq														
4007	AAG	GAG	CCC	GTG	CAC	GGC	GTG	TAC	TAC	GAC	CCC	TCC	AAG	GAC	CTG															
3530															3560															
3527	I	A	E	I	Q	K	Q	G	Q	G	Q	W	T	Y	Q	NL4-3 genbank.SEQ														
3527	ATA	GCA	GAA	ATA	CAG	AAG	CAG	GGG	CAA	GGC	CAA	TGG	ACA	TAT	CAA															
3525	I	A	E	I	Q	K	Q	G	Q	G	Q	W	T	Y	Q	pNL4-3.seq														
3525	ATA	GCA	GAA	ATA	CAG	AAG	CAG	GGG	CAA	GGC	CAA	TGG	ACA	TAT	CAA															
4052	I	A	E	I	Q	K	Q	G	Q	G	Q	W	T	Y	Q	pHDMHgpm2.seq														
4052	ATC	GCC	GAG	ATC	CAG	AAG	CAG	GGC	CAG	GGC	CAG	TGG	ACC	TAC	CAG															
3590																														
3572	I	Y	Q	E	P	F	K	N	L	K	T	G	K	Y	A	NL4-3 genbank.SEQ														
3572	ATT	TAT	CAA	GAG	CCA	TTT	AAA	AAT	CTG	AAA	ACA	GGA	AAA	TAT	GCA															
3570	I	Y	Q	E	P	F	K	N	L	K	T	G	K	Y	A	pNL4-3.seq														
3570	ATT	TAT	CAA	GAG	CCA	TTT	AAA	AAT	CTG	AAA	ACA	GGA	AAA	TAT	GCA															
4097	I	Y	Q	E	P	F	K	N	L	K	T	G	K	Y	A	pHDMHgpm2.seq														
4097	ATC	TAC	CAG	GAG	CCC	TTC	AAG	AAC	CTG	AAG	ACC	GGC	AAA	TAC	GCC															
3620															3650															
3617	R	M	K	G	A	H	T	N	D	V	K	Q	L	T	E	NL4-3 genbank.SEQ														
3617	AGA	ATG	AAG	GGT	GCC	CAC	ACT	AAT	GAT	GTG	AAA	CAA	TTA	ACA	GAG															
3615	R	M	K	G	A	H	T	N	D	V	K	Q	L	T	E	pNL4-3.seq														
3615	AGA	ATG	AAG	GGT	GCC	CAC	ACT	AAT	GAT	GTG	AAA	CAA	TTA	ACA	GAG															
4142	R	M	K	G	A	H	T	N	D	V	K	Q	L	T	E	pHDMHgpm2.seq														
4142	CGC	ATG	AAG	GGC	GCC	CAC	ACC	AAC	GAC	GTG	AAG	CAG	CTG	ACC	GAG															
3680																														
3662	A	V	Q	K	I	A	T	E	S	I	V	I	W	G	K	NL4-3 genbank.SEQ														
3662	GCA	GTA	CAA	AAA	ATA	GCC	ACA	GAA	AGC	ATA	GTA	ATA	TGG	GGA	AAG															
3660	A	V	Q	K	I	A	T	E	S	I	V	I	W	G	K	pNL4-3.seq														
3660	GCA	GTA	CAA	AAA	ATA	GCC	ACA	GAA	AGC	ATA	GTA	ATA	TGG	GGA	AAG															
4187	A	V	Q	K	I	A	T	E	S	I	V	I	W	G	K	pHDMHgpm2.seq														
4187	GCC	GTG	CAG	AAG	ATC	GCC	ACC	GAG	TCC	ATC	GTG	ATC	TGG	GGC	AAG															

Fig. 9F

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Alignment Report of Codon Optimization (pol). MEG, using Clustal method with PAM250 residue weight table

3710															3740															
3707	T	P	K	F	K	L	P	I	Q	F	E	T	W	E	A	NL4-3 genbank.SEQ														
3707	ACT	CCT	AAA	TTT	AAA	TTA	CCC	ATA	CAA	AAG	GAA	ACA	TGG	GAA	GCA															
3705	T	P	K	F	K	L	P	I	Q	F	E	T	W	E	A	pNL4-3.seq														
3705	ACT	CCT	AAA	TTT	AAA	TTA	CCC	ATA	CAA	AAG	GAA	ACA	TGG	GAA	GCA															
4232	T	P	K	F	K	L	P	I	Q	F	E	T	W	E	A	pHDMHgpm2.seq														
4232	ACT	CCC	AAG	TTC	AAG	CTG	CCC	ATC	CAG	AAG	GAG	ACC	TGG	GAG	GCC															
3770																														
3752	W	W	T	E	Y	W	Q	A	T	W	I	P	E	W	E	NL4-3 genbank.SEQ														
3752	TGG	TGG	ACA	GAG	TAT	TGG	CAA	GCC	ACC	TGG	ATT	CCT	GAG	TGG	GAG															
3750	W	W	T	E	Y	W	Q	A	T	W	I	P	E	W	E	pNL4-3.seq														
3750	TGG	TGG	ACA	GAG	TAT	TGG	CAA	GCC	ACC	TGG	ATT	CCT	GAG	TGG	GAG															
4277	W	W	T	E	Y	W	Q	A	T	W	I	P	E	W	E	pHDMHgpm2.seq														
4277	TGG	TGG	ACC	GAG	TAC	TGG	CAG	GCC	ACC	TGG	ATC	CCC	GAG	TGG	GAG															
3800															3830															
3797	F	V	N	T	P	P	L	V	K	L	W	Y	Q	L	E	NL4-3 genbank.SEQ														
3797	TTT	GTC	AAT	ACC	CCT	CCC	TTA	GTG	AAG	TTA	TGG	TAC	CAG	TTA	GAG															
3795	F	V	N	T	P	P	L	V	K	L	W	Y	Q	L	E	pNL4-3.seq														
3795	TTT	GTC	AAT	ACC	CCT	CCC	TTA	GTG	AAG	TTA	TGG	TAC	CAG	TTA	GAG															
4322	F	V	N	T	P	P	L	V	K	L	W	Y	Q	L	E	pHDMHgpm2.seq														
4322	TTC	GTG	AAT	ACC	CCC	CTC	CTG	GTG	AAG	CTG	TGG	TAC	CAG	CTG	GAG															
3860																														
3842	K	E	P	I	I	G	A	E	T	F	Y	V	D	G	A	NL4-3 genbank.SEQ														
3842	AAA	GAA	CCC	ATA	ATA	GGA	GCA	GAA	ACT	TTC	TAT	GTA	GAT	GGG	GCA															
3840	K	E	P	I	I	G	A	E	T	F	Y	V	D	G	A	pNL4-3.seq														
3840	AAA	GAA	CCC	ATA	ATA	GGA	GCA	GAA	ACT	TTC	TAT	GTA	GAT	GGG	GCA															
4367	K	E	P	I	I	G	A	E	T	F	Y	V	D	G	A	pHDMHgpm2.seq														
4367	AAG	GAG	CCC	ATC	ATC	GGC	GCC	GAG	ACC	TTC	TAC	GTG	GAC	GGC	GCC															
3890															3920															
3887	A	N	R	E	T	K	L	G	K	A	G	Y	V	T	D	NL4-3 genbank.SEQ														
3887	GCC	AAT	AGG	GAA	ACT	AAA	TTA	GGA	AAA	GCA	GGA	TAT	GTA	ACT	GAC															
3885	A	N	R	E	T	K	L	G	K	A	G	Y	V	T	D	pNL4-3.seq														
3885	GCC	AAT	AGG	GAA	ACT	AAA	TTA	GGA	AAA	GCA	GGA	TAT	GTA	ACT	GAC															
4412	A	N	R	E	T	K	L	G	K	A	G	Y	V	T	D	pHDMHgpm2.seq														
4412	GCC	AAC	CGC	GAG	ACC	AAG	CTG	GGC	AAG	GCC	GGC	TAC	GTG	ACC	GAC															
3950																														
3932	R	G	R	Q	K	V	V	P	L	T	D	T	T	N	Q	NL4-3 genbank.SEQ														
3932	AGA	GGA	AGA	CAA	AAA	GTT	GTC	CCC	CTA	ACG	GAC	ACA	ACA	AAT	CAG															
3930	R	G	R	Q	K	V	V	P	L	T	D	T	T	N	Q	pNL4-3.seq														
3930	AGA	GGA	AGA	CAA	AAA	GTT	GTC	CCC	CTA	ACG	GAC	ACA	ACA	AAT	CAG															
4457	R	G	R	Q	K	V	V	P	L	T	D	T	T	N	Q	pHDMHgpm2.seq														
4457	CGC	GGC	CGC	CAG	AAG	GTG	GTG	CCC	CTG	ACC	GAC	ACC	ACC	AAC	CAG															

Fig. 9C

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Alignment Report of Codon Optimization (pol) MEG, using Clustal method with PAM250 residue weight table

3980															4010															
3977	K	T	E	L	Q	A	I	H	L	A	L	Q	D	S	G	NL4-3 genbank.SEQ														
3977	AAG	ACT	GAG	TTA	CAA	GCA	ATT	CAT	CTA	GCT	TTG	CAG	GAT	TGG	GGA															
3975	K	T	E	L	Q	A	I	H	L	A	L	Q	D	S	G	pNL4-3.seq														
3975	AAG	ACT	GAG	TTA	CAA	GCA	ATT	CAT	CTA	GCT	TTG	CAG	GAT	TGG	GGA															
4502	K	T	E	L	Q	A	I	H	L	A	L	Q	D	S	G	pHDMHgpm2.seq														
4502	AAG	ACC	GAG	CTG	CAG	GCC	ATC	CAC	CTG	GCC	CTG	CAA	GAC	TCC	GGC															
4040																														
4022	L	E	V	N	I	V	T	D	S	Q	Y	A	L	G	I	NL4-3 genbank.SEQ														
4022	TTA	GAA	GTA	AAC	ATA	GTG	ACA	GAC	TCA	CAA	TAT	GCA	TTG	GGA	ATC															
4020	L	E	V	N	I	V	T	D	S	Q	Y	A	L	G	I	pNL4-3.seq														
4020	TTA	GAA	GTA	AAC	ATA	GTG	ACA	GAC	TCA	CAA	TAT	GCA	TTG	GGA	ATC															
4547	L	E	V	N	I	V	T	D	S	Q	Y	A	L	G	I	pHDMHgpm2.seq														
4547	CTG	GAG	GTG	AAC	ATC	GTG	ACC	GAC	TCC	CAG	TAT	GCA	TTG	GGC	ATC															
4070															4100															
4067	I	Q	A	Q	P	D	K	S	E	S	E	L	V	S	Q	NL4-3 genbank.SEQ														
4067	ATT	CAA	GCA	CAA	CCA	GAT	AAG	AGT	GAA	TCA	GAG	TTA	GTC	AGT	CAA															
4065	I	Q	A	Q	P	D	K	S	E	S	E	L	V	S	Q	pNL4-3.seq														
4065	ATT	CAA	GCA	CAA	CCA	GAT	AAG	AGT	GAA	TCA	GAG	TTA	GTC	AGT	CAA															
4592	I	Q	A	Q	P	D	K	S	E	S	E	L	V	S	Q	pHDMHgpm2.seq														
4592	ATC	CAG	GCC	CAG	CCC	GAC	AAG	TCC	GAG	TCC	GAG	CTG	GTG	TCC	CAG															
4130																														
4112	I	I	E	Q	L	I	K	K	E	K	V	Y	L	A	W	NL4-3 genbank.SEQ														
4112	ATA	ATA	GAG	CAG	TTA	ATA	AAA	AAG	GAA	AAA	GTC	TAC	CTG	GCA	TGG															
4110	I	I	E	Q	L	I	K	K	E	K	V	Y	L	A	W	pNL4-3.seq														
4110	ATA	ATA	GAG	CAG	TTA	ATA	AAA	AAG	GAA	AAA	GTC	TAC	CTG	GCA	TGG															
4637	I	I	E	Q	L	I	K	K	E	K	V	Y	L	A	W	pHDMHgpm2.seq														
4637	ATC	ATC	GAG	CAG	CTG	ATC	AAG	AAG	GAG	AAG	GTG	TAC	CTG	GCC	TGG															
4160															4190															
4157	V	P	A	H	K	G	I	G	G	N	E	Q	V	D	G	NL4-3 genbank.SEQ														
4157	GTA	CCA	GCA	CAC	AAA	GGA	ATT	GGA	GGA	AAT	GAA	CAA	GTA	GAT	GGG															
4155	V	P	A	H	K	G	I	G	G	N	E	Q	V	D	K	pNL4-3.seq														
4155	GTA	CCA	GCA	CAC	AAA	GGA	ATT	GGA	GGA	AAT	GAA	CAA	GTA	GAT	AAG															
4682	V	P	A	H	K	G	I	G	G	N	E	Q	V	D	K	pHDMHgpm2.seq														
4682	GTG	CCC	GCC	CAC	AAG	GGC	ATC	GGC	GGC	AAC	GAG	CAG	GTG	GAC	AAG															
4220																														
4202	L	V	S	A	G	I	R	K	V	L	F	L	D	G	I	NL4-3 genbank.SEQ														
4202	TTG	GTC	AGT	GCT	GGA	ATC	AGG	AAA	GTA	CTA	TTT	TTA	GAT	GGA	ATA															
4200	L	V	S	A	G	I	R	K	V	L	F	L	D	G	I	pNL4-3.seq														
4200	TTG	GTC	AGT	GCT	GGA	ATC	AGG	AAA	GTA	CTA	TTT	TTA	GAT	GGA	ATA															
4727	L	V	S	A	G	I	R	K	V	L	F	L	D	G	I	pHDMHgpm2.seq														
4727	CTG	GTG	TCC	GCC	GGC	ATC	CGC	AAG	GTG	CTG	TTC	CTG	GAC	GGC	ATC															

Fig. 9H

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Alignment Report of Codon Optimization (pol).MEG. using Clustal method with PAM250 residue weight table

4250															4280															
4247	D	K	A	Q	E	E	H	E	K	Y	H	S	N	W	R	NL4-3 genbank.SEQ														
4247	GAT	AAG	GCC	CAA	GAA	GAA	CAT	GAG	AAA	TAT	CAC	AGT	AAT	TGG	AGA	pNL4-3.seq														
4245	D	K	A	Q	E	E	H	E	K	Y	H	S	N	W	R	pHDMHgpm2.seq														
4245	GAT	AAG	GCC	CAA	GAA	GAA	CAT	GAG	AAA	TAT	CAC	AGT	AAT	TGG	AGA															
4772	D	K	A	Q	E	E	H	E	K	Y	H	S	N	W	R															
4772	GAC	AAG	GCC	CAG	GAG	GAG	CAC	GAG	AAG	TAC	CAC	TCC	AAC	TGG	CGC															
4310																														
4292	A	M	A	S	D	F	N	L	P	P	V	V	A	K	E	NL4-3 genbank.SEQ														
4292	GCA	ATG	GCT	AGT	GAT	TTT	AAC	CTA	CCA	CCT	GTA	GTA	GCA	AAA	GAA	pNL4-3.seq														
4290	A	M	A	S	D	F	N	L	P	P	V	V	A	K	E	pHDMHgpm2.seq														
4290	GCA	ATG	GCT	AGT	GAT	TTT	AAC	CTA	CCA	CCT	GTA	GTA	GCA	AAA	GAA															
4817	A	M	A	S	D	F	N	L	P	P	V	V	A	K	E															
4817	GCC	ATG	GCC	TCC	GAC	TTC	AAC	CTG	CCC	CCC	GTG	GTG	GCC	AAG	GAG															
4340															4370															
4337	I	V	A	S	C	D	K	C	Q	L	K	G	E	A	M	NL4-3 genbank.SEQ														
4337	ATA	GTA	GCC	AGC	TGT	GAT	AAA	TGT	CAG	CTA	AAA	GGG	GAA	GCC	ATG	pNL4-3.seq														
4335	I	V	A	S	C	D	K	C	Q	L	K	G	E	A	M	pHDMHgpm2.seq														
4335	ATA	GTA	GCC	AGC	TGT	GAT	AAA	TGT	CAG	CTA	AAA	GGG	GAA	GCC	ATG															
4862	I	V	A	S	C	D	K	C	Q	L	K	G	E	A	M															
4862	ATC	GTG	GCC	TCC	TGC	GAC	AAG	TGC	CAG	CTG	AAG	GGC	GAG	GCC	ATG															
4400																														
4382	H	G	Q	V	D	C	S	P	G	I	W	Q	L	D	C	NL4-3 genbank.SEQ														
4382	CAT	GGA	CAA	GTA	GAC	TGT	AGC	CCA	GGA	ATA	TGG	CAG	CTA	GAT	TGT	pNL4-3.seq														
4380	H	G	Q	V	D	C	S	P	G	I	W	Q	L	D	C	pHDMHgpm2.seq														
4380	CAT	GGA	CAA	GTA	GAC	TGT	AGC	CCA	GGA	ATA	TGG	CAG	CTA	GAT	TGT															
4907	H	G	Q	V	D	C	S	P	G	I	W	Q	L	D	C															
4907	CAC	GGC	CAG	GTG	GAC	TGC	TCC	CCC	GGC	ATC	TGG	CAG	CTG	GAC	TGC															
4430															4460															
4427	T	H	L	E	G	K	V	I	L	V	A	V	H	V	A	NL4-3 genbank.SEQ														
4427	ACA	CAT	TTA	GAA	GGA	AAA	GTT	ATC	TTG	GTA	GCA	GTT	CAT	GTA	GCC	pNL4-3.seq														
4425	T	H	L	E	G	K	V	I	L	V	A	V	H	V	A	pHDMHgpm2.seq														
4425	ACA	CAT	TTA	GAA	GGA	AAA	GTT	ATC	TTG	GTA	GCA	GTT	CAT	GTA	GCC															
4952	T	H	L	E	G	K	V	I	L	V	A	V	H	V	A															
4952	ACC	CAC	CTG	GAG	GGC	AAG	GTG	ATC	CTG	GTG	GCC	GTG	CAC	GTG	GCC															
4490																														
4472	S	G	Y	I	E	A	E	V	I	P	A	E	T	G	Q	NL4-3 genbank.SEQ														
4472	AGT	GGA	TAT	ATA	GAA	GCA	GAA	GTA	ATT	CCA	GCA	GAG	ACA	GGG	CAA	pNL4-3.seq														
4470	S	G	Y	I	E	A	E	V	I	P	A	E	T	G	Q	pHDMHgpm2.seq														
4470	AGT	GGA	TAT	ATA	GAA	GCA	GAA	GTA	ATT	CCA	GCA	GAG	ACA	GGG	CAA															
4997	S	G	Y	I	E	A	E	V	I	P	A	E	T	G	Q															
4997	TCC	GGC	TAC	ATC	GAG	GCC	GAG	GTG	ATC	CCC	GCC	GAG	ACC	GGC	CAG															

Fig. 9I

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Alignment Report of Codon Optimization (pool) MEG, using Clustal method with PAM250 residue weight table

4520															4550															
4517	E	T	A	Y	F	L	L	K	L	A	G	R	W	P	V	NL4-3 genbank.SEQ														
4517	GAA	ACA	GCA	TAC	TTC	CTC	TTA	AAA	TTA	GCA	GGA	AGA	TGG	CCA	GTA															
4515	E	T	A	Y	F	L	L	K	L	A	G	R	W	P	V	pNL4-3.seq														
4515	GAA	ACA	GCA	TAC	TTC	CTC	TTA	AAA	TTA	GCA	GGA	AGA	TGG	CCA	GTA															
5042	E	T	A	Y	F	L	L	K	L	A	G	R	W	P	V	pHDMHgpm2.seq														
5042	GAG	ACC	GCC	TAC	TTC	CTG	CTG	AAG	CTG	GCC	GCC	CGC	TGG	CCC	GTG															
4580																														
4562	K	T	V	H	T	D	N	G	S	N	F	T	S	T	T	NL4-3 genbank.SEQ														
4562	AAA	ACA	GTA	CAT	ACA	GAC	AAT	GGC	AGC	AAT	TTC	ACC	AGT	ACT	ACA															
4560	K	T	V	H	T	D	N	G	S	N	F	T	S	T	T	pNL4-3.seq														
4560	AAA	ACA	GTA	CAT	ACA	GAC	AAT	GGC	AGC	AAT	TTC	ACC	AGT	ACT	ACA															
5087	K	T	V	H	T	D	N	G	S	N	F	T	S	T	T	pHDMHgpm2.seq														
5087	AAG	ACC	GTG	CAC	ACC	GAC	AAC	GGC	TCC	AAC	TTC	ACC	TCC	ACC	ACC															
4610															4640															
4607	V	F	A	A	C	W	W	A	G	I	K	Q	E	F	G	NL4-3 genbank.SEQ														
4607	GTT	AAG	GCC	GCC	TGT	TGG	TGG	GCG	GGG	ATC	AAG	CAG	GAA	TTT	GGC															
4605	V	F	A	A	C	W	W	A	G	I	K	Q	E	F	G	pNL4-3.seq														
4605	GTT	AAG	GCC	GCC	TGT	TGG	TGG	GCG	GGG	ATC	AAG	CAG	GAA	TTT	GGC															
5132	V	K	A	A	C	W	W	A	G	I	K	Q	E	F	G	pHDMHgpm2.seq														
5132	GTG	AAG	GCC	GCC	TGC	TGG	TGG	GCC	GGC	ATC	AAG	CAG	GAG	TTC	GGC															
4670																														
4652	I	P	Y	N	P	Q	S	Q	G	V	I	E	S	M	N	NL4-3 genbank.SEQ														
4652	ATT	CCC	TAC	AAT	CCC	CAA	AGT	CAA	GGA	GTA	ATA	GAA	TCT	ATG	AAT															
4650	I	P	Y	N	P	Q	S	Q	G	V	I	E	S	M	N	pNL4-3.seq														
4650	ATT	CCC	TAC	AAT	CCC	CAA	AGT	CAA	GGA	GTA	ATA	GAA	TCT	ATG	AAT															
5177	I	P	Y	N	P	Q	S	Q	G	V	I	E	S	M	N	pHDMHgpm2.seq														
5177	ATC	CCC	TAC	AAC	CCC	CAG	TCC	CAG	GGC	GTG	ATC	GAG	TCC	ATG	AAC															
4700															4730															
4697	K	E	L	K	K	I	I	G	Q	V	R	D	Q	A	E	NL4-3 genbank.SEQ														
4697	AAA	GAA	TTA	AAG	AAA	ATT	ATA	GGA	CAG	GTA	AGA	GAT	CAG	GCT	GAA															
4695	K	E	L	K	K	I	I	G	Q	V	R	D	Q	A	E	pNL4-3.seq														
4695	AAA	GAA	TTA	AAG	AAA	ATT	ATA	GGA	CAG	GTA	AGA	GAT	CAG	GCT	GAA															
5222	K	E	L	K	K	I	I	G	Q	V	R	D	Q	A	E	pHDMHgpm2.seq														
5222	AAG	GAG	CTG	AAG	AAG	ATC	ATC	GGC	CAA	GTC	CGC	GAC	CAG	GCC	GAG															
4760																														
4742	H	L	K	T	A	V	Q	M	A	V	F	I	H	N	F	NL4-3 genbank.SEQ														
4742	CAT	CTT	AAG	ACA	GCA	GTA	CAA	ATG	GCA	GTA	TTC	ATC	CAC	AAT	TTT															
4740	H	L	K	T	A	V	Q	M	A	V	F	I	H	N	F	pNL4-3.seq														
4740	CAT	CTT	AAG	ACA	GCA	GTA	CAA	ATG	GCA	GTA	TTC	ATC	CAC	AAT	TTT															
5267	H	L	K	T	A	V	Q	M	A	V	F	I	H	N	F	pHDMHgpm2.seq														
5267	CAC	CTG	AAG	ACC	GCC	GTG	CAG	ATG	GCC	GTG	TTC	ATC	CAC	AAC	TTC															

Fig. 9J

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Alignment Report of Codon Optimization (pol) MEG using Clustal method with PAM250 residue weight table.

4790															4820															
4787	K	R	K	G	G	I	G	G	Y	S	A	G	E	R	I	NL4-3 genbank.SEQ														
4787	AAA	AGA	AAA	GGG	GGG	ATT	GGG	GGG	TAC	AGT	GCA	GGG	GAA	AGA	ATA															
4785	K	R	K	G	G	I	G	G	Y	S	A	G	E	R	I	pNL4-3.seq														
4785	AAA	AGA	AAA	GGG	GGG	ATT	GGG	GGG	TAC	AGT	GCA	GGG	GAA	AGA	ATA	-														
5312	K	R	K	G	G	I	G	G	Y	S	A	G	E	R	I	pHDMHgpm2.seq														
5312	AAG	CGC	AAG	GGC	GGC	ATC	GGC	GGC	TAC	TCC	GCC	GGC	GAG	CGC	ATC															
4850																														
4832	V	D	I	I	A	T	D	I	Q	T	K	E	L	Q	K	NL4-3 genbank.SEQ														
4832	GTA	GAC	ATA	ATA	GCA	ACA	GAC	ATA	CAA	ACT	AAA	GAA	TTA	CAA	AAA															
4830	V	D	I	I	A	T	D	I	Q	T	K	E	L	Q	K	pNL4-3.seq														
4830	GTA	GAC	ATA	ATA	GCA	ACA	GAC	ATA	CAA	ACT	AAA	GAA	TTA	CAA	AAA															
5357	V	D	I	I	A	T	D	I	Q	T	K	E	L	Q	K	pHDMHgpm2.seq														
5357	GTG	GAC	ATC	ATC	GCC	ACC	GAC	ATC	CAG	ACC	AAG	GAG	CTG	CAG	AAG															
4880															4910															
4877	Q	I	T	K	I	Q	N	F	R	V	Y	Y	R	D	S	NL4-3 genbank.SEQ														
4877	CAA	ATT	ACA	AAA	ATT	CAA	AAT	TTT	CGG	GTT	TAT	TAC	AGG	GAC	AGC															
4875	Q	I	T	K	I	Q	N	F	R	V	Y	Y	R	D	S	pNL4-3.seq														
4875	CAA	ATT	ACA	AAA	ATT	CAA	AAT	TTT	CGG	GTT	TAT	TAC	AGG	GAC	AGC															
5402	Q	I	T	K	I	Q	N	F	R	V	Y	Y	R	D	S	pHDMHgpm2.seq														
5402	CAG	ATC	ACC	AAG	ATC	CAG	AAC	TTC	CGC	GTG	TAC	TAC	CGC	GAC	TCC															
4940																														
4922	R	D	P	V	W	K	G	P	A	K	L	L	W	K	G	NL4-3 genbank.SEQ														
4922	AGA	GAT	CCA	GTT	TGG	AAA	GGA	CCA	GCA	AAG	CTC	CTC	TGG	AAA	GGT															
4920	R	D	P	V	W	K	G	P	A	K	L	L	W	K	G	pNL4-3.seq														
4920	AGA	GAT	CCA	GTT	TGG	AAA	GGA	CCA	GCA	AAG	CTC	CTC	TGG	AAA	GGT															
5447	R	D	P	V	W	K	G	P	A	K	L	L	W	K	G	pHDMHgpm2.seq														
5447	CGC	GAC	CCC	GTG	TGG	AAG	GGC	CCC	GCC	AAG	CTG	CTG	TGG	AAG	GGC															
4970															5000															
4967	E	G	A	V	V	I	Q	D	N	S	D	I	K	V	V	NL4-3 genbank.SEQ														
4967	GAA	GGG	GCA	GTA	GTA	ATA	CAA	GAT	AAT	AGT	GAC	ATA	AAA	GTA	GTG															
4965	E	G	A	V	V	I	Q	D	N	S	D	I	K	V	V	pNL4-3.seq														
4965	GAA	GGG	GCA	GTA	GTA	ATA	CAA	GAT	AAT	AGT	GAC	ATA	AAA	GTA	GTG															
5492	E	G	A	V	V	I	Q	D	N	S	D	I	K	V	V	pHDMHgpm2.seq														
5492	GAG	GGC	GCC	GTG	GTG	ATC	CAG	GAC	AAC	TCC	GAC	ATC	AAG	GTG	GTG															
5030																														
5010	P	R	R	K	A	K	I	I	R	D	Y	G	K	Q	M	NL4-3 genbank.SEQ														
5010	CCA	AGA	AGA	AAA	GCA	AAG	ATC	ATC	AGG	GAT	TAT	GGA	AAA	CAG	ATG															
5010	P	R	R	K	A	K	I	I	R	D	Y	G	K	Q	M	pNL4-3.seq														
5010	CCA	AGA	AGA	AAA	GCA	AAG	ATC	ATC	AGG	GAT	TAT	GGA	AAA	CAG	ATG															
5537	P	R	R	K	A	K	I	I	R	D	Y	G	K	Q	M	pHDMHgpm2.seq														
5537	CCC	CGC	CGC	AAG	GCC	AAG	ATC	ATC	CGC	GAC	TAC	GGC	AAG	CAG	ATG															

Fig. 9K

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Alignment Report of Codon Optimization (pol.).MEG. using Clustal method with PAM250 residue weight table.

	5060												5090												
5057	A	G	D	D	C	V	A	S	R	Q	D	E	D	..	NL4-3 genbank.SEQ										
5057	GCA	GGT	GAT	GAT	TGT	GTG	GCA	AGT	AGA	CAG	GAT	GAG	GAT	TAA											
5055	A	G	D	D	C	V	A	S	R	Q	D	E	D	..	pNL4-3.seq										
5055	GCA	GGT	GAT	GAT	TGT	GTG	GCA	AGT	AGA	CAG	GAT	GAG	GAT	TAA											
5582	A	G	D	D	C	V	A	S	R	Q	D	E	D	..	pHDMHgpm2.seq										
5582	GCC	GGC	GAC	GAC	TGC	GTG	GCC	TCC	CGC	CAG	GAC	GAG	GAC	TAA											

Fig. 9L

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AGCTTGGCCC	ATTGCATACG	TTGTATCCAT	ATCATAATAT	GTACATTTAT	ATTGGCTCAT	60
GTCCAACATT	ACCGCCATGT	TGACATTGAT	TATTGACTAG	TTATTAATAG	TAATCAATTA	120
CGGGGTCATT	AGTTCATAGC	CCATATATGG	AGTTCCGCGT	TACATAACTT	ACGGTAAATG	180
GCCCGCCTGG	CTGACCGCCC	AACGACCCCC	GCCCATTGAC	GTCAATAATG	ACGTATGTTT	240
CCATAGTAAC	GCCAATAGGG	ACTTTCCATT	GACGTCAATG	GGTGGAGTAT	TTACGGTAAA	300
CTGCCCACCT	GGCAGTACAT	CAAGTGTATC	ATATGCCAAG	TACGCCCCCT	ATTGACGTCA	360
ATGACGGTAA	ATGGCCCCGC	TGGCATTATG	CCCAGTACAT	GACCTTATGG	GACTTTCCCTA	420
CTTGGCAGTA	CATCTACGTA	TTAGTCATCG	CTATTACCAT	GGTGATGCGG	TTTTTGGCAGT	480
ACATCAATGG	GCGTGGATAG	CGGTTTGACT	CACGGGGATT	TCCAAGTCTC	CACCCCATTG	540
ACGTCAATGG	GAGTTTGTTC	TGGCACCAAA	ATCAACGGGA	CTTTCCAAAA	TGTCGTAACA	600
ACTCCGCCCC	ATTGACGCAA	ATGGGCGGTA	GGCGTGTACG	GTGGGAGGTC	TATATAAGCA	660
GAGCTCGTTT	AGTGAACCGT	CAGATCGCCT	GGAGACGCCA	TCCACGCTGT	TTTGACCTCC	720
ATAGAAGACA	CCGGGACCGA	TCCAGCCTCC	CCTCGAAGCT	GATCCTGAGA	ACTTCAGGGT	780
GAGTCTATGG	GACCCCTGAT	GTTTTCTTTC	CCCTTCTTTT	CTATGGTTAA	GTTTCATGTCA	840
TAGGAAGGGG	AGAAGTAACA	GGGTACACAT	ATTGACCAAA	TCAGGGTAAT	TTTGCATTTC	900
TAATTTTAAA	AAATGCTTTC	TTCTTTTAA	ATACTTTTTT	GTTTATCTTA	TTTCTAATAC	960
TTTCCCTAAT	CTCTTTCTTT	CAGGGCAATA	ATGATACAAT	GTATCATGCC	TCTTTGCACC	1020
ATTCTAAAGA	ATAACAGTGA	TAATTTCTGG	GTTAAGGCAA	TAGCAATATT	TCTGCATATA	1080
AATATTTCTG	CATATAAATT	GTAACGTATG	TAAGAGGTTT	CATATTGCTA	ATAGCAGCTA	1140
CAATCCAGCT	ACCATTCTGC	TTTTATTTTA	TGGTTGGGAT	AAGGCTGGAT	TATTCTGAGT	1200
CCAAGCTAGG	CCCTTTTGCT	AATCATGTTC	ATACCTCTTA	TCTTCTCCTC	ACAGCTCCTG	1260
GGCAACGTGC	TGGTCTGTGT	GCTGGCCCAT	CACTTTGGCA	AAGAATTCTA	GACTGCCATG	1320
GGCGCCCGCG	CCTCCGTGCT	GTCCGGCGGC	GAGCTGGACA	AGTGGGAGAA	GATCCGCCTG	1380
CGCCCCGGCG	GCAAGAAGCA	GTACAAGCTG	AAGCACATCG	TGTGGGCCTC	CCGCGAGCTG	1440
GAGCGCTTCG	CCGTGAACCC	CGGCCCTGCTG	GAGACCTCCG	AGGGCTGCCG	CCAGATCCTG	1500
GGCCAGCTGC	AGCCCTCCCT	GCAAACCGGC	TCCGAGGAGC	TGCGCTCCCT	GTACAACACC	1560
ATCGCCGTGC	TGTACTGCGT	GCACCAGCGC	ATCGACGTGA	AGGACACCAA	GGAGGCCCTG	1620
GACAAGATCG	AGGAGGAGCA	GAACAAGTCC	AAGAAGAAGG	CCCAGCAGGC	CGCCGCCGAC	1680
ACCGGCAACA	ACTCCCAGGT	GTCCCAGAAC	TACCCCATCG	TGCAGAACCT	GCAGGGCCAG	1740
ATGGTGCACC	AGGCCATCTC	CCCCCGCACC	CTGAACGCCCT	GGGTGAAGGT	GGTGGAGGAG	1800
AAGGCCCTTCT	CCCCCGAAGT	CATCCCCATG	TTCTCCGCCC	TCTCCGAGGG	CGCCACCCCC	1860
CAGGACCTGA	ACACCATGCT	GAACACCGTG	GGCGGCCACC	AGGCCGCCAT	GCAGATGCTG	1920
AAGGAGACCA	TCAACGAGGA	GGCCGCCGAG	TGGGACCGCC	TGCACCCCGT	GCACGCCGCG	1980
CCCATCGCCC	CCGGCCAGAT	GCGCGAGCCC	CGCGGCTCCG	ACATCGCCGG	CACCACCTCC	2040
ACCCTGCAAG	AGCAGATCGG	CTGGATGACC	CACAACCCCC	CCATCCCCGT	GGGCGAGATC	2100
TACAAGCGCT	GGATCATCCT	GGGCCTGAAC	AAGATCGTGC	GCATGTACTC	CCCCACCTCC	2160
ATCCTGGACA	TCCGCCAGGG	CCCCAAGGAG	CCCTTCCGCG	ACTACGTGGA	CCGCTTCTAC	2220
AAGACCCCTGC	GCGCCGAGCA	GGCCTCCCAG	GAGGTAAAGA	ACTGGATGAC	CGAGACCCTG	2280
CTGGTGCAGA	ACGCCAATCC	CGACTGCAAG	ACCATCCTGA	AGGCCCTGGG	CCCCGGCGCC	2340
ACCCTGGAGG	AGATGATGAC	CGCCTGCCAG	GGCGTGGCG	GCCCCGGCCA	CAAGGCCCGC	2400
GTGCTGGCCG	AGGCCATGTC	CCAAGTCACC	AACCCCGCCA	CCATCATGAT	CCAGAAGGGC	2460
AACTTCCGCA	ACCAGCGCAA	GACCGTGAAG	TGCTTCAACT	GCGGCAAGGA	GGGCCACATC	2520
GCCAAGAACT	GCCGCGCCCC	CCGCAAGAAG	GGCTGCTGGA	AGTGCGGCAA	GGAGGGCCAC	2580
CAGATGAAAG	ATTGTAATGA	GAGACAGGCT	AATTTTTTAG	GGAAGATCTG	GCCTTCCCAC	2640
AAGGGAAGGC	CAGGGAATTT	TCTTCAGAGC	AGACCAGAGC	CAACAGCCCC	ACCAGAAGAG	2700
AGCTTCAGGT	TTGGGGAAGA	GACAACAAC	CCCTCTCAGA	AGCAGGAGCC	GATAGACAAG	2760
GAAGTGTATC	CTTTAGCTTC	CCTCAGATCA	CTCTTTGGCA	GCGACCCCTC	GTCACAATAA	2820

Fig. 10A

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AGATCGGTGG	CCAGCTGAAG	GAGGCGCTGC	TGGACACCGG	CGCCGACGAC	ACCGTGCTGG	2880
AGGAGATGAA	CCTGCCCGGC	CGGTSSAAGC	CDAAGATGAT	CGCGGGCATC	GGCGGCTTCA	2940
TCAAAGTCCG	CCAGTACGAC	CAGATCTCTGA	TGGAGATCTG	CGCCACAAAG	GCCATCGGCA	3000
CCGTGCTGGT	GGGCCCCAC	CCGTGSAACA	TGATCGGCGG	CAACCTGCTG	ACCCAGATCG	3060
GCTGCACCGT	GAACCTCCCC	ATCTTCCCCA	TGGAGACCGT	GCCTCGTGAAG	CTGAAGCCCG	3120
GCATGGACGG	CCCCAAAGTC	AAGCAGTGGC	CTCTGACCGA	GGAGAAGATC	AAGGCCCTGG	3180
TGGAGATCTG	CACCGAGATG	GAGAAGGAGG	GCAAGATCTC	CAAGATCGGC	CCCGAGAACC	3240
CCTACAACAC	CCCCGTGTTT	GCCATCAAGA	AGAAGGACTC	CACCAAGTGG	CGCAAGCTGG	3300
TGGACTTCCG	CGAGCTGAAC	AAGCGCACCG	AGGACTTCTG	GGAGGTGCAG	CTGGGCATCC	3360
CCCACCCCGC	CGGCCTGAAG	CAGAAGAAAGT	CGGTGACCGT	GCTGGACGTG	GGCGACGCCT	3420
ACTTCTCCGT	GGCCCTGGAC	AAGGACTTCC	GCAAGTACAC	CGCCTTCACC	ATCCCTTCCA	3480
TCAACAACGA	GACCCCCGGC	ATCCGCTACC	AGTACAAAGT	GCTGCCCCAG	GGCTGGAAGG	3540
GCTCCCCCGC	CATCTTCCAG	TGCTCCATGA	CCAAGATCTT	GGAGCCCTTC	CGCAAGCAGA	3600
ACCCCGACAT	CGTGATCTAC	CAGTACATGS	ACGACCTGTA	CGTGGGCTCC	GACCTGGAGA	3660
TCGGCCAGCA	CCGCACCAAG	ATCGAGGAGT	TGCGCCAGCA	CCTGCTGCGC	TGGGGCTTCA	3720
CCACCCCGCA	CAAGAAGCAC	CAGAAGGAGC	CCCCCTTCCT	GTGGATGGGC	TACGAGCTGA	3780
ACCCCGACAA	GTGGACCGTG	CAGCCCATCG	TGCTGCCCGA	GAAGGACTCC	TGGAGCCGTG	3840
ACGACATCCA	GAAGCTGGTG	GGCAAGCTGA	ACTGGGCTTC	CCAGATCTAC	CCCGGCATCA	3900
AAGTCCGCCA	GCTGTGCAAG	CTGCTGCGCG	GCACCAAGGC	CCTGACCGAG	GTGGTGCCCC	3960
TGACCGAGGA	GGCCGAGCTG	GAGCTGGCCG	AGAACCGCGA	GATCCTGAAG	GAGCCCGTGC	4020
ACGGCGTGTA	CTACGACCCC	TCCAAGGACC	TGATCGCGGA	GATCCAGAAG	CAGGGCCAGG	4080
GCCAGTGGAC	CTACCAGATC	TACCAGGAGC	CCTTCAAGAA	CCTGAAGACC	GGCAAATACG	4140
CCCGCATGAA	GGGCGCCAC	ACCAACGACG	TGAAGCACT	GACCGAGGCC	GTGCAGAAGA	4200
TGCCACCCGA	GTCCATCGTG	ATCTGGGGCA	AGACTCCCAA	GTTCAGCTG	CCCATCCAGA	4260
AGGAGACCTG	GGAGGCCTGG	TGGACCGAGT	ACTGGCAGGC	CACCTGGATC	CCCGAGTGGG	4320
AGTTCGTGAA	CACCCCCCCC	CTGGTGAAGC	TGTGTACCA	GCTGGAGAAG	GAGCCCATCA	4380
TCGGCGCCGA	GACCTTCTAC	GTGGACGGCG	CGGCCAAGCG	CGAGACCAAG	CTGGGCAAGG	4440
CCGGCTACGT	GACCGACCGC	GGCCGCCAGA	AGGTGGTGCC	CCTGACCGAC	ACCACCAACC	4500
AGAAGACCGA	GCTGCAGGCC	ATCCACCTGG	CCCTGCAAGA	CTCCGGCCTG	GAGGTGAACA	4560
TCGTGACCGA	CTCCCAGTAT	GCATTGGGCA	TCATCCAGGC	CCAGCCCGAC	AAGTCCGAGT	4620
CCGAGCTGGT	GTCCCAGATC	ATCGAGCAGC	TGATCAAGAA	GGAGAAGGTG	TACCTGGCCT	4680
GGGTGCCCGC	CCACAAGGGC	ATCGGCGGCA	ACGAGCAGGT	GGACAAGCTG	GTGTCCGCCG	4740
GCATCCGCAA	GGTGTGTTC	CTGGACGGCA	TCGACAAGGC	CCAGGAGGAG	CACGAGAAGT	4800
ACCACTCCAA	CTGGCGCGCC	ATGGGCTCCG	ACTTCAACCT	GCCCCCGTGG	GTGGCCAAGG	4860
AGATCGTGGC	CTCCTGCGAC	AAGTGCCAGC	TGAAGGGCGA	GGCCATGCAC	GGCCAGGTGG	4920
ACTGCTCCCC	CGGCATCTGG	CAGCTGGACT	GCACCCACCT	GGAGGGCAAG	GTGATCCTGG	4980
TGGCCGTGCA	CGTGGCCTCC	GGCTACATCG	AGGCCGAGGT	GATCCCCGCC	GAGACCGGCC	5040
AGGAGACCGC	CTACTTCCTG	CTGAAGCTGG	CGGCCGCTG	GCCCCGTGAAG	ACCGTGCACA	5100
CCGACAACGG	CTCCAACCTC	ACCTCCACCA	CCGTGAAGGC	CGCCTGCTGG	TGGGCCGGCA	5160
TCAAGCAGGA	GTTCCGGCATC	CCCTACAACC	CCCAGTCCCA	GGGCGTGATC	GAGTCCATGA	5220
ACAAGGAGCT	GAAGAAGATC	ATCGGCCAAG	TCCGCGACCA	GGCCGAGCAC	CTGAAGACCG	5280
CCGTGCAGAT	GGCCGTGTTT	ATCCACAACCT	TCAAGCGCAA	GGGCGGCATC	GGCGGCTACT	5340
CCGCCGGCGA	GCGCATCTGT	GACATCATCG	CCACCGACAT	CCAGACCAAG	GAGCTGCAGA	5400
AGCAGATCAC	CAAGATCCAG	AACTTCCGCG	TGTACTACCG	CGACTCCCGC	GACCCCGTGT	5460
GGAAGGGCCC	CGCCAAGCTG	CTGTGGAAGG	GCGAGGGCGC	CGTGGTGATC	CAGGACAACCT	5520
CCGACATCAA	GGTGGTGCCC	CGCCGCAAGG	CCAAGATCAT	CCGCGACTAC	GGCAAGCAGA	5580
TGGCCGGCGA	CGACTGCGTG	GCCTCCCGCC	AGGACGAGGA	CTAACACATG	GAAAAGATTA	5640

Fig. 10B

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GTAAAACACC	ATAGGCCGCT	CTAGAGGATC	CAAGCTTATC	GATACCGTCG	ACCTCGAGGG	5700
CCCAGATCTA	ATTCACCCCA	CCAAGTGCAGG	CTGCCTATCA	GAAAGTGGTG	GCTGGTGTGG	5760
CTAATGCCCT	GGCCCCACAAG	TATCACTAAG	CTCGCTTTCT	TGCTGTCCAA	TTTCTATTAA	5820
AGGTTCCCTT	GTTCCCTAAG	TCCAACACT	AAACTGGGGG	ATATTATGAA	GGGCCTTGAG	5880
CATCTGGATT	CTGCCTAATA	AAAAACATTT	ATTTTCATTG	CAATGATGTA	TTTAAATTAT	5940
TTCTGAATAT	TTTACTAAAA	AGGGAATGTG	GGAGGTCAGT	GCATTTAAAA	CATAAAGAAA	6000
TGAAGAGCTA	GTTCAAACCT	TGGGAAAATA	CACATATATCT	TAACTCCAT	GAAAGAAGGT	6060
GAGGCTGCAA	ACAGCTAATG	CACATTGGCA	ACAGCCCTG	ATGCCTATGC	CTTATTCATC	6120
CCTCAGAAAA	GGATTCAAGT	AGAGGCTTGA	TTTGGAGGTT	AAAGTTTTC	TATGCTGTAT	6180
TTTACATTAC	TTATTGTTTT	AGCTGTCTCT	ATGAATGTCT	TTTCACTACC	CATTTGCTTA	6240
TCCTGCATCT	CTCAGCCTTG	ACTCCACTCA	GTTCTCTTGC	TTAGAGATAC	CACCTTTCCC	6300
CTGAAGTCTT	CCTTCCATGT	TTTACGGCGA	GATGGTTTCT	CCTCGCTGG	CCACTCAGCG	6360
TTAGTTGTCT	CTGTTGTCTT	ATAGAGGTCT	ACTTGAAGAA	GGAAAAACAG	GGGGCATGGT	6420
TTGACTGTCC	TGTGAGCCCT	TCTTCCCTGC	CTCCCCACT	CACAGTGACC	CGGAATCCCT	6480
CGACATGGCA	GTCTAGATCA	TTCTTGAAGA	CGAAAGGGCC	TCGTGATACG	CCTATTTTTA	6540
TAGGTTAATG	TCATGATAAT	AATGGTTTCT	TAGACGTCAG	GTGGCACTTT	TCGGGGAAAT	6600
GTGCGCGGAA	CCCCTATTTG	TTTATTTTTC	TAAATACATT	CAAATATGTA	TCCGCTCATG	6660
AGACAATAAC	CCTGATAAAT	GCTTCAATAA	TATTGAAAAA	GGAAGAGTAT	GAGTATTCAA	6720
CATTTCCTGT	TCGCCCTTAT	TCCCTTTTTT	GCGGCATTTT	GCCTTCCTGT	TTTTGCTCAC	6780
TCAGAAACGC	TGGTGAAAGT	AAAAGATGCT	GAAGATCAGT	TGGGTGCACG	AGTGGGTTAC	6840
ATCGAATCGG	ATCTCAACAG	CGGTAAGATC	CTTGAGAGTT	TTCGCCCCGA	AGAACGTTTT	6900
CCAATGATGA	GCACTTTTAA	AGTTCTGCTA	TGTGGCGCGG	TATTATCCCG	TATTGACGCC	6960
GGGCAAGAGC	AACTCGGTCT	CCGCATACAC	TATTCTCAGA	ATGACTTGGT	TGAGTACTCA	7020
CCAGTCACAG	AAAAGCATCT	TACGGATGGC	ATGACAGTAA	GAGAAATTATG	CAGTGTGCCC	7080
ATAACCATGA	GTGATAACAC	TGCGGCCAAC	TTACTTCTGA	CAACGATCGG	AGGACCGAAG	7140
GAGCTAACCG	CTTTTTTGCA	CAACATGGGG	GATCATGTAA	CTCGCCTTGA	TCGTGGGGAA	7200
CCGGAGCTGA	ATGAAGCCAT	ACCAAACGAC	GAGCGTGACA	CCACGATGCC	TGTAGCAATG	7260
GCAACAACGT	TGCGCAAAC	ATTAAGTGGC	GAACACTTAA	CTCTAGCTTC	CCGGCAACAA	7320
TTAATAGACT	GGATGGAGGC	GGATAAAGTT	GCAGGACCAC	TTCTGCGCTC	GGCCCTTCCG	7380
GCTGGCTGGT	TTATTGCTGA	TAAATCTGGA	GCCGGTGAGC	GTGGGTCTCG	CGGTATCATT	7440
GCAGCACTGG	GGCCAGATGG	TAAGCCCTCC	CGTATCGTAG	TTATCTACAC	GACGGGGAGT	7500
CAGGCAACTA	TGGATGAACG	AAATAGACAG	ATCGCTGAGA	TAGGTGCCTC	ACTGATTAAG	7560
CATTGGTAAC	TGTCAGACCA	AGTTTACTCA	TATATACTTT	AGATTGATTT	AAAACCTCAT	7620
TTTTAATTTA	AAAGGATCTA	GGTGAAGATC	CTTTTTGATA	ATCTCATGAC	CAAAATCCCT	7680
TAACGTGCGT	TTTCGTTCCA	CTGAGCGTCA	GACCCCGTAG	AAAAGATCAA	AGGATCTTCT	7740
TGAGATCCTT	TTTTTCTGCG	CGTAATCTGC	TGCTTGCAAA	CAAAAAAACC	ACCGCTACCA	7800
GCGGTGGTTT	GTTTGCCGGA	TCAAGAGCTA	CCAACCTCTT	TTCCGAAGGT	AACTGGCTTC	7860
AGCAGAGCGC	AGATACCAAA	TACTGTTCTT	CTAGTGTAGC	CGTAGTTAGG	CCACCACTTC	7920
AAGAAGCTCT	TAGCACCGCC	TACATACCTC	GCTCTGCTAA	TCCTGTTACC	AGTGGCTGCT	7980
GCCAGTGGCG	ATAAGTCGTG	TCTTACCGGG	TTGGACTCAA	GACGATAGTT	ACCGGATAAG	8040
GCGCAGCGGT	CGGGCTGAAC	GGGGGGTTTC	TGCACACAGC	CCAGCTTGGA	GCGAACGACC	8100
TACACCGAAC	TGAGATACCT	ACAGCGTGAG	CTATGAGAAA	GCGCCACGCT	TCCCGAAGGG	8160
AGAAAGGCGG	ACAGGTATCC	GGTAAGCGGC	AGGGTCGGAA	CAGGAGAGCG	CACGAGGGAG	8220
CTTCCAGGGG	GAAACGCCTG	GTATCTTTAT	AGTCCTGTCT	GTTTCGCCA	CCTCTGACTT	8280
GAGCGTCGAT	TTTTGTGATG	CTCGTCAGGG	GGGCGGAGCC	TATGGAAAAA	CGCCAGCAAC	8340
GGATGCGCCG	CGTGCGGCTG	CTGGAGATGG	CGGACGCGAT	GGATATGTTC	TGCCAAGGGT	8400
TGTTTTCGCG	ATTCACAGTT	CTCCGCAAGA	ATTGATTGGC	TCCAATTCTT	GGAGTGGTGA	8460

Fig. 10C

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ATCCGTTAGC	GAGGTGCCGC	CGGCTTCCAT	TCAGGTCGAG	GTGGCCCGGC	TCCATGCACC	8520
GCGACGCAAC	GCGGGGAGGC	AGACAAGGTA	TAGGGCGGCG	CCTACAATCC	ATGCCAACCC	8580
GTTCCATGTG	CTCGCCGAGG	CGGCATAAAT	CCCCGTGACG	ATCAGCGGTC	CAATGATCGA	8640
AGTTAGGCTG	GTAAGAGCCG	CGAGCGATCC	TTGAAGCTGT	CCCTGATGGT	CGTCATCTAC	8700
CTGCCCTGGAC	AGCATGGCCT	GCAACGCGGG	CATCCCGATG	CCGCCGGAAG	CGAGAAGAAT	8760
CATAATGGGG	AAGGCCATCC	AGCCTCGCGT	CGGGGAGCTT	TTTGCAAAAG	CCTAGGCCTC	8820
CAAAAAAGCC	TCCTCACTAC	TTCTGGAATA	GCTCAGAGGC	CGAGGCGGCC	TCGGCCTCTG	8880
CATAAATAAA	AAAAATTAGT	CAGCCATG	8908			

Fig. 10D

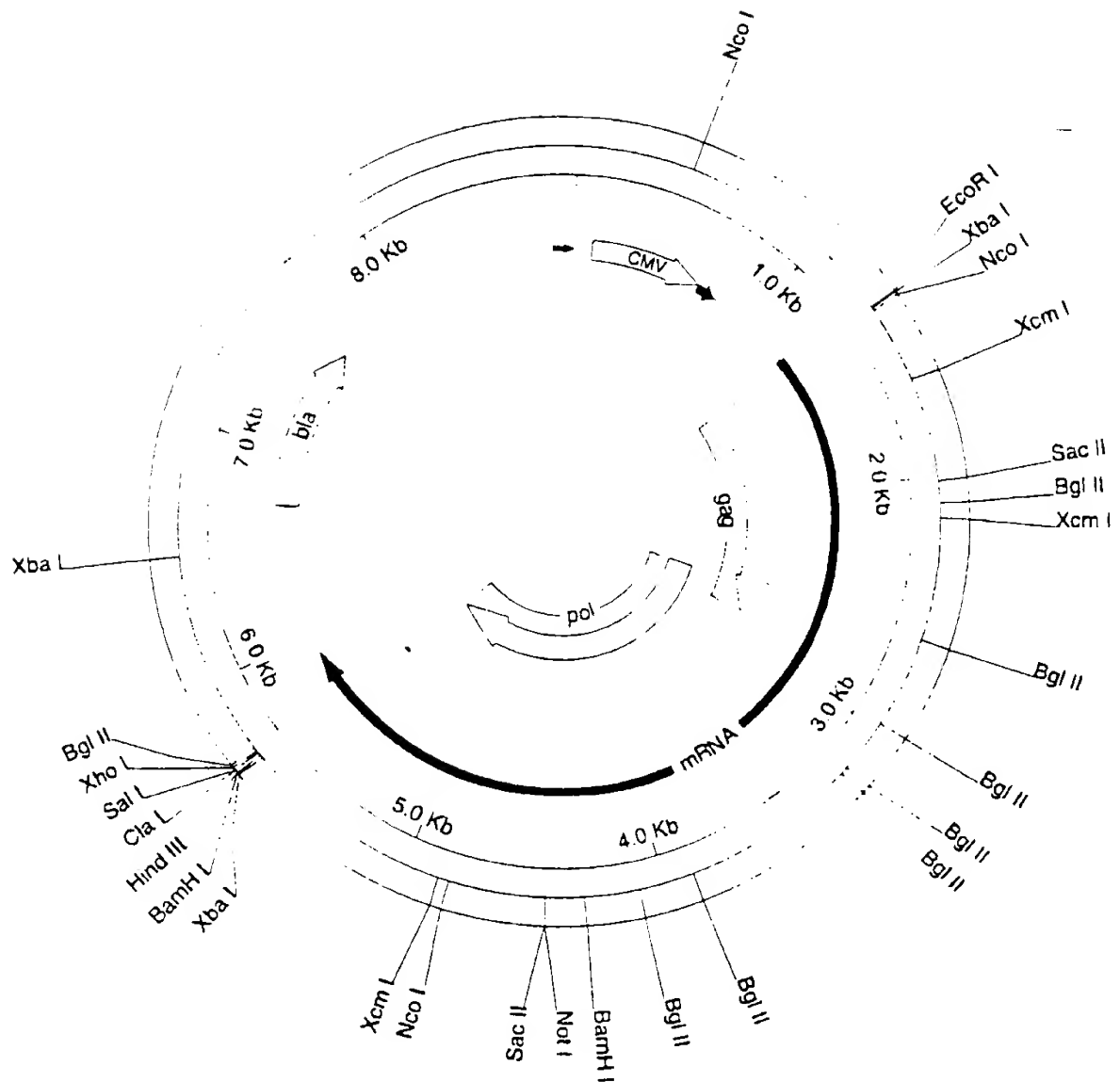


Fig. 11

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/20675

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/86 C12N5/10 C12N7/04 C12N15/49 C07K14/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NALDINI L ET AL: "IN VIVO GENE DELIVERY AND STABLE TRANSDUCTION OF NONDIVIDING CELLS BY A LENTIVIRAL VECTOR" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 272, no. 5259, 12 April 1996 (1996-04-12), pages 263-267, XP000583652 ISSN: 0036-8075 cited in the application the whole document</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-4

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search

25 February 2000

Date of mailing of the international search report

03/03/2000

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Authorized officer

Chambonnet, F

INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 99/20675

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
A	<p>HASELHORST D ET AL: "STABLE PACKAGING CELL LINES AND HIV-1 BASED RETROVIRAL VECTOR SYSTEMS" GENE THERAPY,GB,MACMILLAN PRESS LTD., BASINGSTOKE, vol. 1, no. SUPPL. 02, 18 November 1994 (1994-11-18), page S14 XP002063698 ISSN: 0969-7128 the whole document</p>	---
A	<p>ST LOUIS D ET AL: "CONSTRUCTION AND CHARACTERIZATION OF HIV-1 RETROVIRAL VECTORS AND REPLICATION-DEFECTIVE HIV-1 PACKAGING CELL LINES" INTERNATIONAL CONFERENCE ON AIDS AND THE STD WORLD CONGRESS,XX,XX, 1 June 1993 (1993-06-01), page 244 XP002063695 the whole document</p>	1
A	<p>CARROLL R ET AL: "A HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)-BASED RETROVIRAL VECTOR SYSTEM UTILIZING STABLE HIV-1 PACKAGING CELL LINES" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 68, no. 9, 1 September 1994 (1994-09-01), pages 6047-6051, XP002063697 ISSN: 0022-538X the whole document</p>	1
X	<p>HOLLER T P ET AL: "HIV1 INTEGRASE EXPRESSED IN ESCHERICHIA COLI FROM A SYNTHETIC GENE" GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 136, 22 December 1993 (1993-12-22), pages 323-328, XP000199775 ISSN: 0378-1119 the whole document</p>	39
A	<p>ANDRE S ET AL: "INCREASED IMMUNE RESPONSE ELICITED BY DNA VACCINATION WITH A SYNTHETIC GP120 SEQUENCE WITH OPTIMIZED CODON USAGE" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 72, no. 2, 1 February 1998 (1998-02-01), pages 1497-1503, XP002073767 ISSN: 0022-538X the whole document</p>	39-43